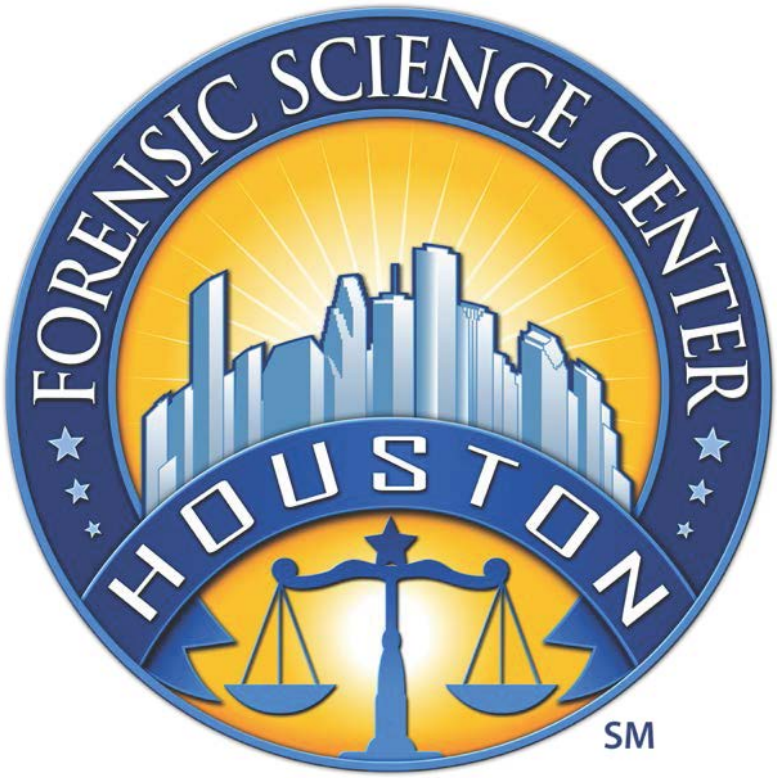
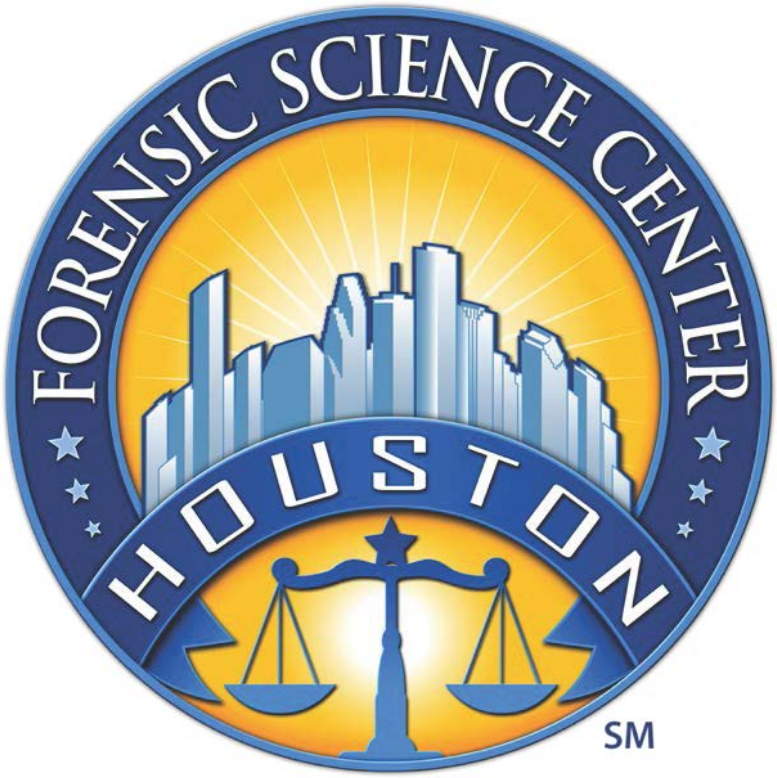


Forensic Biology: Screening
Standard Operating Procedures

Forensic Analysis Division

Table of Contents

1. Overview
2. Facilities
   2.1 Work Areas
   2.2 Contamination
   2.3 Safety
3. Equipment Quality Control and Maintenance
   3.1 Maintenance and Calibration
   3.2 Equipment
4. Quality Assurance
   4.1 Chemical and Reagent Labels
   4.2 Quality Control Checks
   4.3 Logbook
   4.4 Proficiency Testing
5. Evidence Evaluation and Handling
   5.1 Case Acceptance and Evaluation
   5.2 Evidence Handling
6. Blood Detection
   6.1 Phenolphthalein Test Kit
   6.2 Luminol
   6.3 ABAcard® HemaTrace® Test Devices
   6.4 References
7. Semen Detection
   7.1 Alternate Light Source
   7.2 Acid Phosphatase Spot Test
   7.3 Body Fluid Extraction
   7.4 Microscopic Spermatozoa Examination
   7.5 SERATEC® PSA (p30) Semiquant Test
   7.6 Guidelines for Sexual Assault Cases When Contact or Digital Penetration is Alleged
   7.7 References
8. Trace Observation/Collection
9. Contact DNA Collection
10. Portioning for DNA Analysis
11. Case Records
    11.1 Examination Documentation
    11.2 Reports
    11.3 Examination Counts
12. Abbreviations
13. Chelex Extraction of DNA
14. Plexor HY Quantification



Forensic Biology: Screening Overview

Forensic Analysis Division

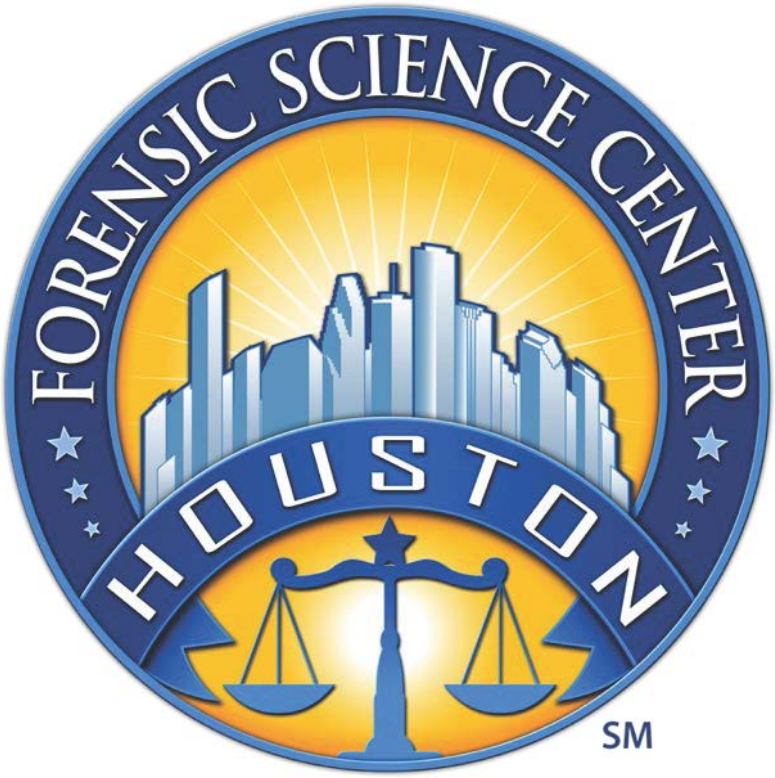
Overview

The Biology Standard Operating Procedure (SOP) manual specifies procedures for routine examinations and analyses of biological evidence for human identification. It is approved for use in the Biology section of the laboratory. Within the scope of that purpose, it is intended to ensure effective and efficient use of the laboratory facilities for the benefit of all user agencies. It incorporates the quality assurance elements necessary to ensure the reliability and uniformity of analyses and reported conclusions.

Each approved revision of this manual will be version-controlled and archived for retrieval by date of authority. Only the approved revision in effect at the time of analysis governs the analysis. The official version of this controlled document shall be published on the laboratory's intranet webpage and can be viewed from network computers. Any deviation from accepted protocol requires approval as outlined in the Methods section of the quality manual, and must be documented in the case file.

This SOP is only one part of the policies and procedures that govern all work performed by the Biology section. The Biology section will follow the requirements detailed herein, in addition to the following:

- Parent Agency General Orders/Policy
- Laboratory Quality Manual
- Laboratory Safety Manual
- Laboratory Biology Training Manual



Forensic Biology: Screening Facilities

Forensic Analysis Division

2 Facilities

The Biology section will be designed to provide adequate space and setup to perform casework analysis. General facility requirements are described in the Quality Manual.

2.1 Work Areas

The Biology section will have space for evidence examination. The tasks performed in this area can include: blood and semen fluid identification, swab collections, cutting of stains, portioning of swabs and cuttings, microscopy and trace evidence collection.

2.2 Contamination

With the facility provided, setup and procedures will be designed to minimize the potential for DNA contamination. Traffic in and through areas in which testing occurs will be minimized.

Prevention and Decontamination

10% bleach, or DNA Away where bleach may be harmful to instruments or equipment, will be used to decontaminate utensils and workbenches. Other commercial decontaminants may be used only if they have been demonstrated to destroy or inactivate DNA.

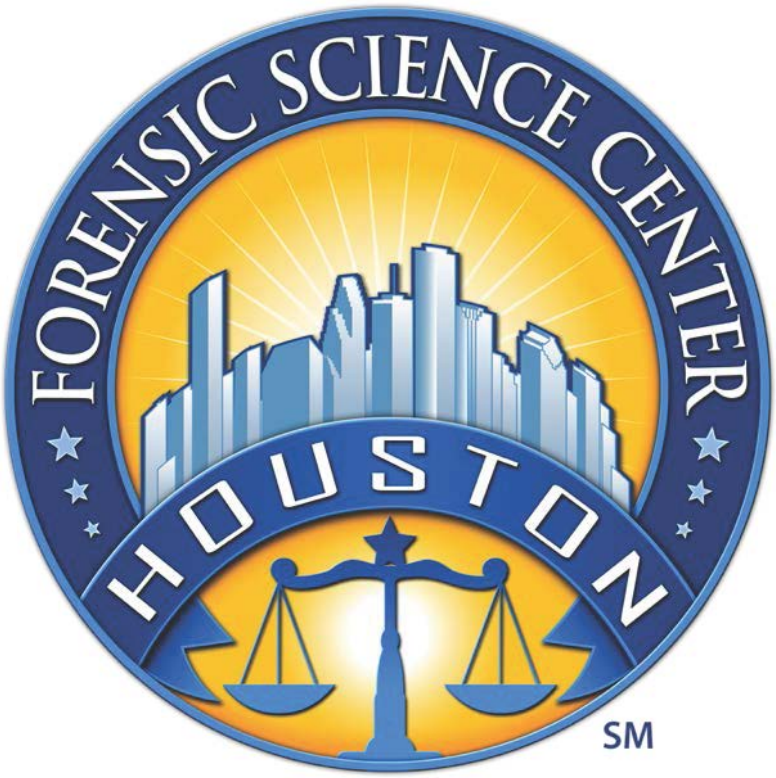
Wear disposable gloves during all testing and reagent preparation. Change gloves frequently and whenever gloves may have become contaminated. Discard gloves when leaving a work area. Use sterile, disposable pipette tips and microcentrifuge tubes. Change pipette tips between samples. Centrifuge all liquid to the bottom of closed microcentrifuge tubes before opening.

Clean work surfaces in the evidence examination areas thoroughly with a lab-approved decontaminant prior to and at the end of each evidence examination session. Use disposable bench paper whenever possible and change each time it comes in contact with evidence. Use a clean cutting surface such as weighing paper or piece of paper for evidence. Protect supplies of this paper from dust and other particulates or aerosols. Clean instruments (e.g., scalpels, scissors, forceps) with decontaminant between evidence samples. To prevent contamination of other standards or evidence, handle each piece of evidence one at a time. Liquid samples, such as blood standards, and wet exhibits shall be allowed to air dry in a method that it will not come into contact with other pieces of evidence.

Wear gloves when cleaning glassware and plastics. In general, clean glassware/plastics with an appropriate soap, e.g., Liquinox or Alconox, and water. Rinse with deionized or distilled water and allow to air-dry inverted. Glassware should be autoclaved. Plastic ware should be placed under UV light for ~15 minutes. Store glassware and plastic ware after being autoclaved or being cleaned by UV light.

2.3 Safety

There are biological and chemical hazards in the laboratory. Each lab employee is responsible for familiarity with the Laboratory Safety Manual. Any incident or condition that occurs in or under the control of the laboratory and that threatens the immediate or future health of any individual must be immediately brought to the attention of the section supervisor and a laboratory safety officer. Corrective action related to safety incidents will be defined by laboratory management.



Forensic Biology: Screening
Equipment Quality Control and Maintenance

Forensic Analysis Division

3 Equipment Quality Control and Maintenance

3.1 Maintenance and Calibration

In order to provide and maintain the quality of the work provided in the Biology section, it is necessary to ensure laboratory equipment is in good working order. Routine quality control and maintenance accomplishes this. The calibration intervals listed below are generally considered to be the minimum appropriate in each case, provided that the equipment is of good quality and of proven stability and the laboratory has both the equipment capability and expertise to perform adequate internal checks. More frequent checks are acceptable. If there is any question concerning the reliability of an instrument or piece of equipment, a maintenance check should be performed immediately.

Full maintenance, calibration records, and repair activities shall be maintained and recorded in an equipment calibration and maintenance log. This log will include at a minimum: the date, activity, laboratory personnel performing or overseeing the activity, non-crime laboratory technician(s) performing or overseeing the activity, and a record of quality control checks performed to verify operation prior to returning a piece of equipment to casework use. The section supervisor and QA Manager are responsible for ensuring all systems are checked and maintained as required. Whenever practical, equipment that requires calibration will be labeled with the date when last calibrated and the date or expiration criteria when recalibration is due.

Critical equipment is any piece of equipment that must be maintained in a proper working order to ensure the reliability of results produced. Some critical equipment and associated maintenance requirements can be found in the Quality Manual. Additional equipment and maintenance requirements are included here.

Equipment Operation Manuals

Equipment operation manuals will be readily available to each examiner approved to use the equipment.

3.2 Equipment

Ultrasonic Cleaner

Ultrasonic cleaners use ultrasonic energy in the form of sound waves to create a mechanical “scrubbing” action to loosen debris from all surfaces that the solution touches.

This equipment’s condition is routinely maintained for the Biology section’s procedures. The water should be clear and clean with no evidence of bacterial/fungal growth or rust. The following procedure should be performed as needed: Wash the inside of the ultrasonic cleaner with detergent and rinse well with water. Fill bath with the appropriate quantity of diH_2O water.

Mini-centrifuges

Mini-centrifuges are bench top, unrefrigerated centrifuges that have been designed for **quick spin-downs from tube walls and caps** of tubes. These mini-centrifuges are equipped with a circular, one sized rotor. The maximum speed is 6000 rpm. The relative centrifugal force can be determined as outlined in the manufacturer’s instructions, if required.

Please consult the appropriate equipment manual for specific instructions on maintenance and operation of the mini-centrifuge. These centrifuges will primarily be used for removing liquid from the cap of a microcentrifuge tube. They will not be used for pelleting cellular material.

Thermometers

All thermometers must be either calibrated or their accuracy must be verified to ensure the temperature is being accurately measured.

NIST-traceable thermometers must be purchased or re-certified at least once every two years. A NIST-traceable thermometer certified for two years and used for conducting performance checks on equipment shall require the annual performance check. A NIST-traceable thermometer certified for two years that is not used for conducting performance checks does not require the annual performance checks and may be used until the certification expires. A NIST-traceable thermometer to be used beyond its certification date shall be recertified or be subject to the annual performance check requirements.

The minimum requirements of a performance check of a thermometer used for performing performance checks may be accomplished: (1) through certification by an outside vendor; or (2) in-house by the comparison of one or more temperature readings at various time intervals against another NIST-traceable thermometer.

Thermometers that are not NIST-traceable must be verified annually by comparison against a NIST-traceable thermometer. Any deviation from the NIST-traceable thermometer will be noted and all other readings adjusted by the same amount, and in the same direction for each thermometer. If the deviation is greater than 3°C, the thermometer should be serviced or replaced.

Thermometers should be handled carefully to avoid breakage. Thermometers may be wiped clean to facilitate easy and accurate readings. If any discontinuities are detected, repair or replace the thermometer.

Autoclaves

Autoclaves are used to sterilize solutions, glassware, and instruments by subjecting them to high pressure and high heat simultaneously. Autoclaves also may be used to sterilize biohazard trash prior to discarding. Please consult the instructions on the outside of the unit for specific information on operation of the autoclave.

Whenever an item is autoclaved, a small piece of autoclave tape must be adhered to the item, to verify correct functioning of the autoclave. Use proper sterilizer-loading procedures when placing materials in sterilizer chamber. Those include: all solid containers/ instruments must be placed so that water or air will not be trapped in them and sufficient space must be left between items to allow steam circulation. The temperature gauge should read 120^0C at the start and during the appropriate cycle.

If the autoclave is not functioning properly, a qualified service technician may repair the instrument.

Alternate Light Source (ALS) Machines

ALS machines are used to presumptively identify semen in forensic casework. The fan air filter, optical filters, and lenses should be cleaned on a regular basis (approximately every 6 months), where applicable. Please consult the appropriate equipment manuals for more specific instructions on maintenance and operation of the ALS machines.

Swab Drying Box E025

Swab drying boxes are used to dry swabs created in the lab during forensic casework. Before and after each use, the box should be wiped inside and out with a 10% bleach solution or other lab-approved decontaminant and dried with a soft cloth. Do not autoclave, immerse, or abrade the unit.

Refrigerators and Freezers

Please refer the Laboratory Quality Manual for refrigerator and freezer maintenance. Reagents and evidence will be stored separately, either in separate cooling units or in separate space within those units.

Pipettes

Volumetric pipettes are used to accurately and precisely measure the volume of a solution to be delivered. The accuracy of the measurement is dependent upon the correct technique of the user as well as the proper maintenance and performance of the pipette. The pipette should be able to provide accuracy, precision, repeatability and reproducibility over the full range of the pipette's capabilities. The Biology section is supplied with pipettes that cover the volume range from 0.1 - 1000 µl. Calibration will be performed at least annually on these instruments. When a pipette is determined to be performing improperly, it will be returned to the manufacturer, or another qualified repair technician, so that the problem may be identified and corrected.

Quality control data should include the type of pipette, volume range, model, and serial number.

Procedure

1. Set the volume of the pipette using the knob in the pipetting push-button.
2. Firmly attach the appropriate tip (per manufacturer's recommendations) to the pipette.
3. Press the push-button to the first positive stop.
4. Holding the pipette vertically, immerse the tip into the sample liquid per manufacturer's recommended depth.
5. Release the push-button slowly and smoothly to aspirate the sample.
6. Pausing for a moment, withdraw the tip from the liquid.
7. Depending on the sample, it may be necessary to pre-rinse the tip before aspirating the sample.
8. To dispense the sample, place the tip into the transfer vessel and press the push-button to the first stop.
9. Pausing for a moment, press the push-button to the second stop to 'blowout' or expel any remaining liquid.
10. Slowly, release the push-button
11. Eject the tip by pressing the ejector button.

Performance Verification/Maintenance

1. Each pipette should be externally calibrated and certified by an approved calibration vendor annually.

2. Internal pipette verifications should be performed about 6 months after the external calibration.

3. Pipette performance verification should be documented.

4. To check for accuracy, room temperature deionized water should be pipetted into a weighing vessel on an analytical balance.

5. The pipette should be checked using 3-points over the full range with 5 replicates at each volume.

6. Each pipette should fall within the verification tolerance ranges according to Addendum 3A before it may be used for casework.

7. If a pipette fails a performance verification check, it should be recalibrated. Calibrations are not performed in-house.

8. Follow manufacturer's instructions for troubleshooting maintenance if needed.

9. If an analyst has reason that a pipette is not working properly they must:

    a. Perform a pipette verification and if the pipette is not in proper working order:

    b. Clearly mark the pipette "OUT OF SERVICE".

    c. Inform the section manager. No laboratory case work will be performed using the pipette until the problem is corrected.

    d. Repair or send out the pipette for repairs

    e. Verify that the pipette is working correctly and falls with-in the proper tolerances following routine repair, maintenance or calibration.

    f. Maintain the appropriate documentation and update the log book.

Water Filtration System

Water is used in reagents that are prepared in the laboratory. Therefore, it is necessary to ensure only high quality, reagent grade water is being utilized. Generally, 15MΩ-cm or greater deionized water is sufficient for the reagents utilized in our laboratory.

Water quality should be checked on a weekly basis. The following procedure will be utilized:

1. Turn on the deionized water.
2. Allow water to run for 1-2 minutes.

3. Record both the MΩ-cm reading and the temperature reading displayed on the resistivity monitor in the Water Quality logbook.
4. If reading is below 15MΩ-cm, call for service. A qualified service technician may repair the system. Label the system as being “out of service” until the appropriate service is performed and the instrument is functioning properly.

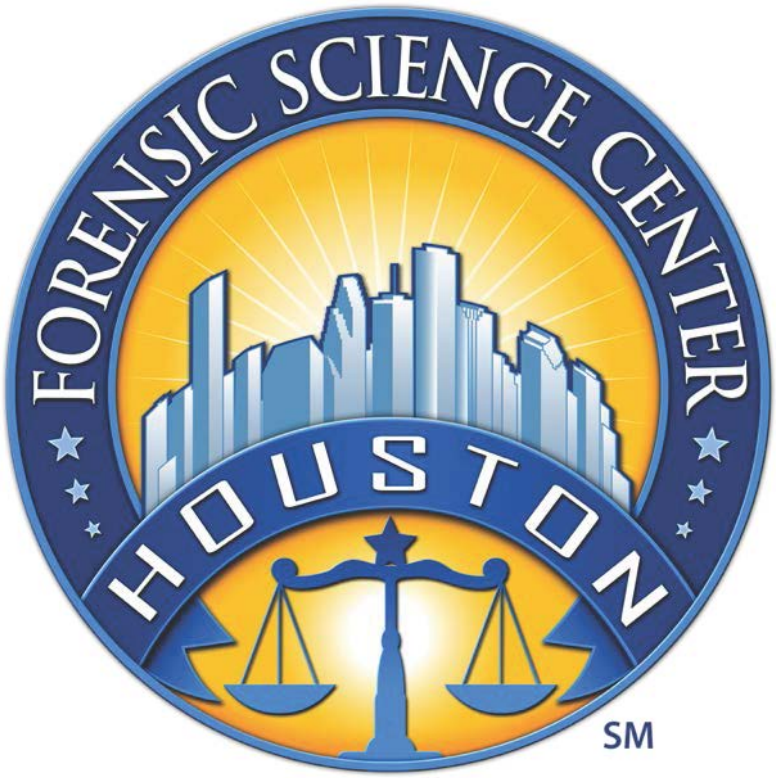
Balances

Please refer to the Laboratory Quality Manual for the balance calibration schedule.

Addendum 3A

Volumetric Pipette Range	Percent	Check Volume (uL)	Acceptable Check (uL) Average needs to be between these values	
0.5-10	5	2.5	2.375	2.625
	3	7.5	7.275	7.725
	3	10	9.7	13
1-10	5	2.5	2.375	2.375
	3	7.5	7.275	7.725
	3	10	9.7	10.3
5-50	3	12.5	12.125	12.875
	3	37.5	36.375	38.625
	3	50	48.5	51.5
2-20	3	5	4.85	5.15
	3	15	14.55	15.45
	3	20	19.4	20.6
10-100	3	25	24.25	25.75
	3	75	72.75	77.25
	3	100	97	103
20-100	3	25	24.25	25.75
	3	75	72.75	77.25
	3	100	97	103
20-200	3	50	48.5	51.5
	3	150	145.5	154.5
	3	200	194.0	206
40-200	3	50	48.5	52
	3	150	145.5	154.5
	3	200	194.0	206
50-200	3	50	48.5	51.5
	3	150	145.5	154.5
	3	200	194	206
100-1000	3	250	242.5	257.5
	3	750	727.5	772.5
	3	1000	970	1030

200-1000	3	250	242.5	257.5
	3	750	727.5	772.5
	3	1000	970	1030



Forensic Biology: Screening

Quality Assurance

Forensic Analysis Division

4 Quality Assurance

In order to provide and maintain the quality of the work provided in the Biology section, it is necessary to identify certain reagents as critical. Critical reagents are those that require testing prior to use on evidentiary samples in order to prevent unnecessary loss of sample. All reagent preparations and quality control testing must be documented on the appropriate worksheet or log.

If any critical reagent does not pass all quality control checks, it may not be utilized in casework. All inconsistencies will be documented and reported to the Section Supervisor. Problems that cannot be resolved must be reported to the manufacturer.

Reagents and supplies that have passed their expiration dates may not be used on casework samples. Outdated reagents may be used for training purposes only, but must be clearly marked as such.

4.1 Chemical and Reagent Labels

Purchased chemicals and reagents will be marked on the container with the date received and/or date opened. In general, the manufacturer's labeling will be followed to determine expiration dates of purchased chemicals and reagents. If no manufacturer expiration exists for a purchased reagent, it will be considered expired 5 years from the date received and should be marked on the container.

Prepared reagent labels will include the reagent name, initials of preparer and expiration date. In general, most solutions prepared in the Biology section shall expire 1 year from the date of preparation. However, the expiration date of the overall reagent will be no later than the expiration date of the individual reagent with the nearest expiration date (unless otherwise specified). Additional information may be documented in a reagent log. The lot number contains the date prepared and the initials of the preparer, e.g. mmddyyinitials.

4.2 Quality Control Checks

The following shall be tested to confirm that they are working properly with a positive and negative control each day used:

- Acid Phosphatase Spot Test
- Alternate Light Source
- Luminol mixture
- Phenolphthalein test kit

The following shall be quality control checked to confirm they are working properly with each new lot received:

- ABAcard® HemaTrace® kits
- Phosphate Buffered Saline (1X)
- SERI Christmas Tree Stain

- SERATEC® PSA Semiquant test devices

4.2.1 Acid Phosphatase (AP) Spot Test

Scope

AP Spot Test solution undergoes a color change (Brentamine reaction) in the presence of acid phosphatase, which is found in highest concentration in semen. Instructions for use and interpretation are in the AP Spot Test procedure.

Safety

This reagent presents the following hazards: may cause cancer; irritant to eyes, respiratory system and skin; and corrosive (can cause burns). Wear gloves, lab coat, and mask during preparation and use. Broken skin should be covered.

Equipment and Supplies

- AP Spot Test premixed powder – purchased
- Darkened or foil wrapped container for storage
- Scale
- Sterile diH_2O
- Timer
- Known semen sample
- Known non-semen sample

Preparation

1. Measure 10mL of sterile diH_2O in an appropriate container.
2. Add 0.26g of the purchased AP Spot Test premixed powder.
3. Mix until AP Spot Test premixed powder is completely dissolved in water.

   Note: Volume may be smaller or larger than 10mL, but the amount of AP Spot Test premixed powder added should be adjusted accordingly.

Prior to use each day, the prepared AP solution must be subjected to the appropriate in-house quality control test as outlined below:

1. A known semen sample will be used as a positive control. This in-house-prepared sample is typically presented on filter paper.
2. An untreated piece of filter paper or swab moistened with sterile diH_2O will be used as a negative control. Ideally, the negative control substrate mimics your sample substrate as closely as possible.
3. Apply ~ 1 drop of prepared AP solution to both controls.
4. Observe samples for 60 seconds for a color change.
5. The known semen sample must give a strong purple color change indicating a positive result.

6. The untreated filter paper or sterile swab must have no color change indicating a negative result.

The results of each control test must be recorded on every applicable serology worksheet.

Storage, Labeling, and Expiration

Store prepared solution in a darkened or foil wrapped container. Label with reagent name, lot number, and expiration date. Reconstituted reagent is stable and sensitive for one day's use – make solution fresh daily. Store purchased powder frozen. Purchased powder may be used on casework samples up to the expiration date stated on the bottle.

4.2.2 Alternate Light Source

Scope

An Alternate Light Source is specially designed for detection of forensic stains, fibers, and fingerprints. Instructions for use and interpretation are in the Alternate Light Source procedure.

Safety

This instrument presents the following hazards: exposing the skin to the beam of light (directly from the unit) can cause burns and other skin damage. It is essential that proper eye protection be provided. Avoid looking directly at the light source, reflected, or refracted light. Remove all unnecessary reflective surfaces from exam room. Wear goggles, gloves, lab coat, and other proper laboratory attire.

Mini-Crimescope MCS-400 ALS: If the bulb fails (suddenly stops emitting light), it may leak vapors for a few minutes. To be safe, immediately leave the room and let the unit run (fans) for 5-10 minutes until vapors are vented.

Equipment and Supplies

- ALS set at 400 or 455 nm
- Orange and/or yellow glasses
- Known semen sample
- Known non-semen sample

Prior to use each day, the ALS must be subjected to the appropriate in-house quality control test as outlined below:

1. Turn on power switch and ensure the fans are working.
2. Wear the proper glasses.
3. Turn on lamp switch.
4. Darken the examination room.
5. Direct the ALS at the samples.
6. The known semen sample must fluoresce indicating a positive result.
7. The known non-semen sample must not fluoresce indicating a negative result.

When ready to shut down the ALS, turn off lamp switch and wait for the unit to cool down (~5 minutes). Then turn off the power switch.

The results of each control test must be recorded on every applicable serology worksheet.

Storage and Expiration

Store equipment in a clean, dry atmosphere. The bulb should be replaced after 3-3.5 years of non-regular usage.

4.2.3 Luminol

Scope

Catalytic tests for blood are based on the peroxidase-like activity exhibited by the heme group of hemoglobin. In the case of luminol, the catalytic oxidation of the substrate compound produces light. Instruction for use and interpretation are in the Luminol procedure.

Safety

This reagent presents the following hazards: luminol is an irritant. Sodium perborate and sodium carbonate are toxic and irritating. Avoid breathing dust; do not get in eyes, on skin, or on clothing. Avoid breathing sprayed solution. Wash hands after handling. Gloves must be worn during testing. Broken skin should be covered.

Equipment and Supplies

- Luminol powder: 3-aminophthalhydrazide
- Sodium carbonate
- Sodium perborate
- Sterile diH_2O
- Two darkened or foil wrapped spray bottles
- Scale
- Known blood sample
- Known non-blood sample

Preparation (Reagent A)

1. Measure 250 mL of diH_2O.
2. Add 2.5 g of sodium carbonate.
3. Mix until all of the powder is dissolved.
4. Add 0.5 g of luminol powder.
5. Mix until all of the powder is dissolved.

Preparation (Reagent B)

1. Measure 250 mL of diH_2O.
2. Add 6.5 g of sodium perborate**.**
3. Mix until all of the powder is dissolved.

Mix equal volumes of Reagents A and B to create a luminol mixture. The mixture is unstable and must be prepared fresh immediately before use; it is only stable for about one hour.

Prior to use, the luminol mixture must be subjected to the appropriate in-house quality control test as outlined below:

1. Darken the examination room as completely as possible.
2. Spray luminol mixture onto a known blood sample (prepared in-house).
3. Spray luminol mixture onto a known non-blood sample (cotton swab or filter paper).
4. Observe samples for immediate luminescence.
5. The known blood sample must give an immediate luminescence indicating a positive result.
6. The cotton swab or filter paper must not produce luminescence indicating a negative result.

The results of each control test must be recorded on every applicable serology worksheet.

Storage, Labeling, and Expiration

The luminol powder, sodium carbonate and sodium perborate should be stored in a cool, dry, well-ventilated area away from incompatible substances. The expirations for reagents A and B (stored separately) are 8 weeks from date solutions were prepared. If stored, the labels must include, at a minimum, "Luminol, Reagent A" or "Luminol, Reagent B" as appropriate, initials, the date prepared, and the expiration date. Prior to each use, check the reagents for precipitate. If a precipitate has formed in either reagent, ensure that the precipitate is back in solution before use. Luminol mixture should be labeled with the appropriate lot number and expiration date. The mixture will expire one hour after use.

4.2.4 Phenolphthalein Test Kit

Scope

PHT Test Kit Solutions B and C are used for presumptive blood identification by oxidizing phenolphthalin (colorless in a basic solution such as the test reagent) to phenolphthalein (pink) in the presence of heme and hydrogen peroxide. Instructions for use and interpretation are in the phenolphthalein test kit procedure.

Safety

This reagent presents the following hazards:

Solution B: Flammable. Handle with care. Harmful or fatal if swallowed, inhaled, or absorbed through skin. Avoid contact with eyes, skin, and clothing. Light sensitive! Avoid light, heat, sparks, and flame. Keep container tightly closed.

Solution C: Handle with care. May be harmful if swallowed, inhaled, or absorbed through skin. Avoid contact with eyes, skin, and clothing. Avoid heat, sparks, and flame. Keep container tightly closed.

Wear gloves, lab coat, and mask during preparation and use. Broken skin should be covered.

Equipment and Supplies

- Solution B
- Solution C
- Sterile diH_2O
- Sterile cotton swab(s)
- Known blood sample
- Known non-blood sample

Prior to use each day, PHT Solutions B and C must be subjected to the appropriate in-house quality control test as outlined below:

1. Rub a cotton swab moistened with sterile diH_2O on a known bloodstain sample. This swab will be used as a positive control.
2. An untreated cotton swab moistened with sterile diH_2O will be used as a negative control.
3. Apply ~1 drop of Solution B to each swab.
4. Apply ~1 drop of Solution C to each swab.
5. Observe the swabs for a color change.
6. The cotton swab with the known bloodstain sample must give an immediate pink color change indicating a positive result.
7. The untreated cotton swab must have no color change indicating a negative result.

The results of each control test must be recorded on every applicable serology worksheet.

Storage, Labeling, and Expiration

PHT test kits can be stored at room temperature. Each analyst will have an aliquot of Solutions B and C that will be placed in amber bottles stored at room temperature or refrigerated. Each bottle will be labeled with appropriate reagent name, lot numbers, and expiration dates. The kits may be used on casework samples up to the expiration date stated on the bottles.

4.2.5 ABAcard® HemaTrace® Test Devices

Scope

ABAcard® HemaTrace® test kits are used to determine the presence of human blood in suspected blood stains. Instructions for use and interpretation are in the ABAcard® HemaTrace® procedure.

Safety

Wear gloves, lab coat, mask, and other proper laboratory attire. Broken skin should be covered.

Equipment and Supplies

- ABAcard® HemaTrace® test device(s)
- ABAcard® HemaTrace® extraction buffer
- Pipette and pipette tips
- Known blood sample
- Timer

Each new lot of ABAcard® HemaTrace® test kits must be subjected to the appropriate in-house quality control test as outlined below:

1. Make a $1{:}10^6$ dilution from a known human blood sample in extraction buffer provided in kit.
2. Add 150µl of dilution to sample well “S” of one test device to be used as a positive control.
3. Add 150 µl of extraction buffer from kit to sample well of second test device to be used as a negative control.
4. Read results up to 10 minutes from application of sample.
5. The $1{:}10^6$ dilution of known human blood must give a positive result. The extraction buffer provided in the kit must give a negative result.

Storage, Labeling, and Expiration

The test devices must be stored at room temperature (below 82ºF) and must remain in the sealed pouch until use. Label each test device with the name of the appropriate sample used. The test kits may be used on casework samples up to the expiration date stated on the box.

4.2.6 Phosphate Buffered Saline (1X)

Scope

PBS is used as an extraction agent for serological stains. Instructions for use and interpretation are in the Phosphate Buffered Saline procedure.

Safety

This reagent presents the following hazards: causes severe eye, respiratory tract and skin irritation. Avoid contact with skin and clothing. Wear gloves, lab coat, and other proper laboratory attire. Broken skin should be covered.

Equipment and Supplies

- OmniPur 10X PBS Premixed Powder
- diH_2O
- Graduated cylinder
- Two sterile containers for storage

Preparation (10X)

1. Measure 1 liter of diH_2O.
2. Add 100g of 10X PBS Premixed Powder.
3. Mix until all of the powder is dissolved.
4. Autoclave solution.

Preparation (1X)

1. Measure 100mL of 10X PBS solution.
2. Measure 900 mL of sterile diH_2O.
3. Mix 10X PBS and sterile diH_2O.

Each new lot of 1X PBS must be subjected to an appropriate in-house quality control test with the lot of SERATEC® PSA Semiquant test currently in use. See SERATEC® PSA Semiquant test quality control procedure for further detail.

Storage, Labeling, and Expiration

1X and 10X PBS solutions can be stored at room temperature. Label each bottle with appropriate concentration, lot number and expiration date. Expiration for both PBS concentrations is one year from the date 10X PBS solution was prepared. Both concentrations of PBS may be used on casework samples up to this expiration date.

4.2.7 SERI Christmas Tree Stain

Scope

SERI Christmas Tree Stain is a Kernechtrot-Picroindigocarmine differential biological stain that assists in scanning slides for the presence of spermatozoa. The test consists of a two stain method which dyes sperm heads red, tails green and epithelium green or blue with red nuclei. Instructions for use and interpretation are in the SERI Christmas Tree Stain procedure.

Safety

This reagent presents the following hazards: May cause irritation upon contact. Toxic. Do not swallow. Do not inhale. Avoid contact with skin and eyes. Wear gloves and proper laboratory attire. Broken skin should be covered.

Equipment and Supplies

- Microscope slide
- Known semen sample
- Pipette and pipette tips
- Heat block
- Christmas Tree Stain - Solution A and Solution B
- Timer
- Tap water
- 95% ethanol
- Compound microscope

Each new lot of SERI Christmas Tree Stain must be subjected to the appropriate in-house quality control test as outlined below:

1. Pipette 10 µl of a neat in-house sample onto a glass slide. Fix smear by heating the slide on the heat block until dry.
2. Stain the slide.
    a. Cover the sample area on the slide with Solution A for 10 minutes.
    b. Wash with tap water by gentle flooding.
    c. Cover the sample area with Solution B for 15 seconds.
    d. Wash with tap water by gentle flooding.
    e. Flood the slide with 95% ethanol and allow the slide to dry for at least 5 minutes on the heat block.
3. Examine the slide for the presence of spermatozoa at 1000X magnification. Sperm heads must be stained red, and any observed tails must be stained green indicating a positive result.

Storage and Expiration

The stain must be stored at room temperature. The stain may be used on casework samples up to the expiration date stated on the bottles.

4.2.8 SERATEC® PSA Semiquant Test Devices

Scope

SERATEC® PSA Semiquant test devices are used to indicate the possible presence of p30 in suspected semen stains. Instructions for use and interpretation are in SERATEC® PSA Semiquant test procedure.

Safety

Wear gloves, lab coat, mask, and other proper laboratory attire. Broken skin should be covered.

Equipment and Supplies

- SERATEC® PSA Semiquant test device(s)
- 1X PBS
- Pipette and pipette tips
- Known semen sample
- Timer

Each new lot of SERATEC® PSA Semiquant Test devices must be subjected to the appropriate in-house quality control test as outlined below:

1. Make a $1:10^5$ dilution from a known semen sample in 1X PBS.
2. Add 120 μl of dilution to sample well of one test device to be used as a positive control.
3. Add 120 μl of 1X PBS to sample well of second test device to be used as a negative control.
4. Read results up to 10 minutes from application of sample.
5. The $1:10^5$ dilution of known semen must give a positive result. The 1X PBS must give a negative result.
6. Mark the stock solution bottle of 1X PBS with all lot numbers of SERATEC® PSA Semiquant test devices quality control checked with that lot of 1X PBS.

Storage, Labeling, and Expiration

The test devices must be stored at room temperature and must remain in the sealed pouch until use. Label each test device with the name of the appropriate sample used. The test devices may be used on casework samples up to the expiration date stated on the sealed pouch.

4.3 Logbook

Reagent preparation logs should include the lot number, the reagent name, the initials of preparer, and the expiration date. Quality control logs for ABAcard® HemaTrace® kits, Phosphate Buffered Saline (1X), SERI Christmas Tree Stain, and SERATEC® PSA Semiquant tests will also contain the test date, signature of the analyst performing the quality control, a second reader signature, if applicable, and date and any supporting documentation necessary to demonstrate the reagent met all of the standards.

4.4 Proficiency Testing

Proficiency testing and review will follow the requirements of the quality manual. In addition, the Quality Manager will maintain a copy of the analysis documentation for each proficiency test. Proficiency tests will be analyzed and interpreted according to standard operating procedures including technical review. Proficiency test participants will be notified of their final test results.

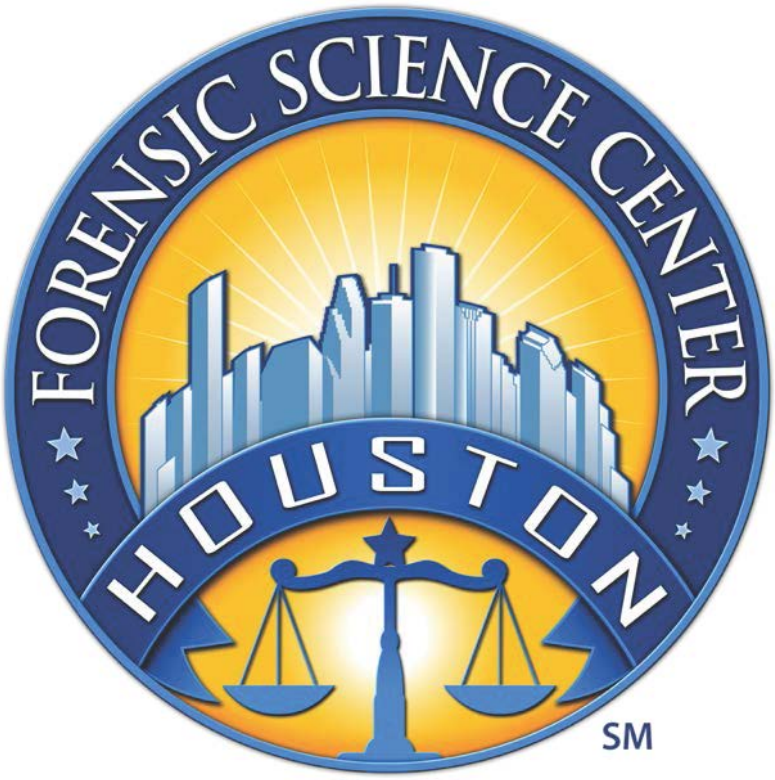
Analysts will enter into a proficiency test program within 6 months of being deemed competent on any portion of casework analysis. Proficiency testing should include each technology to the full extent to which analysts and technicians participate in casework.

Proficiency work is to follow as closely as possible that of normal casework. If symbols are used in the reporting of data to the proficiency testing agency, they must be defined in the results submission form. It is also advisable to include comments in the comments section of the proficiency results form to explain certain results. For example, if a result is reported as inconclusive, the reason should be presented in the comments section.

During the case file reviews, the proficiency results form (including the screening data and comments sections), along with the case file, shall be reviewed by both the technical and administrative reviewers to ensure proper transcription of results by the author of the results from.

If performing quantification for proficiency samples, the analyst should create his/her own DNA standards.

Only one proficiency results form will be completed for proficiency tests to ensure that only the most complete form is submitted to the proficiency testing provider in order to be included in the provider's published external summary report. The screening analyst shall complete the proficiency test results form if the test does not proceed to DNA analysis; the DNA analyst shall complete the proficiency test results form if the test does proceed to DNA analysis.



Forensic Biology: Screening Evidence Evaluation and Handling

Forensic Analysis Division

5 Evidence Evaluation and Handling

The Biology section provides body fluid identification (semen and blood) and performs the collection and preservation of trace evidence and possible contact DNA evidence.

5.1 Case Acceptance and Evaluation

Refer to the Quality Manual for procedures regarding the submission of evidence into the Crime Laboratory. Before a case is worked, the case and the requested examinations will be evaluated. The examiner should be aware of the requested examinations, and when possible, the reason(s) for the requested analyses and the quality and quantity of the evidence.

In order to expedite casework, with the exception of sexual assault kits, it is recommended that 5-10 items of evidence should initially be screened for cases containing large volumes of evidence. Emphasis should be placed on those items believed to be of the most significant evidentiary value after consultation with the submitting party. Of the items screened, it is recommended that a maximum of 5 items of evidence continue on to DNA analysis initially. Additional items may be analyzed at a later time. Individual case needs may dictate that the initial quantity of examined items is greater than the recommended range presented above.

When submitted in conjunction with a sexual assault kit, clothing evidence does not typically have to be examined if the kit components yield sufficiently positive samples. ("Sufficiently positive" is fluid and relevant to the case information.) Clothing should be examined if the corresponding sexual assault evidence kit does not reveal any positive probative evidence, if there are multiple suspects, or there is a recent consensual sexual partner. Good judgment using the case information and/or the client's specific requests should always supersede this guideline.

5.2 Evidence Handling

It is not possible to anticipate every situation that may arise or to prescribe a specific course of action for every case; therefore, the examiner must exercise good judgment based on experience and common sense. In some cases, the manual offers guidelines for analysis that must be tempered with the experience of the examiner. However, any portion of a procedure not explicitly qualified as a guideline may not be modified for use in casework without prior written approval as outlined in the Quality Manual.

On a sexual assault case, for example, the analyst will have a choice of using conventional serology for the detection of semen or quantitative PCR for the detection of male DNA. The evidence type may warrant one method over another. It may be more informative to detect actual spermatozoa on a non-intimate garment that may have been collected from a common area than to detect male DNA; alternatively, the detection of male DNA via quantitative PCR on an intimate swab may be more probative to a particular case.

When ample sample permits, the analyst should consider retesting when "inconclusive" results are obtained.

Please also refer to the Quality Manual for the Handling of Evidence (5.8).

Universal Precautions

Body fluids and extracts may contain infective agents. All analysts should routinely use appropriate barrier precautions to prevent skin and mucous membrane exposure when in contact with any body fluids. Gloves should be worn and changed after contact with each piece of evidence. Masks, lab coats, and protective eyewear should be worn during procedures that anticipate contact with body fluids to prevent exposure of mucous membranes of the mouth, nose, and eyes. All analysts should take precautions to prevent injuries caused by scalpels or other sharp instruments or devices during procedures, when cleaning, or during disposal. After sharp instruments are used, they should be placed in a puncture-resistant container for disposal.

Evidence Requiring Latent Print Examination

Submitting parties may request that items be analyzed for latent prints as well as biological material. In general biological evidence examinations precede any latent print examination. Appropriate steps should be taken, including consultation with the submitting party and/or a latent prints examiner, to ensure the appropriate course of action for the evidence presented. In some cases, it may be best if one type of examination is chosen over another. If an analyst finds a distinct print during examination, appropriate steps should be taken to preserve the print and inform the officer.

Storage of Evidence

Biological evidence must be properly stored to preserve biochemicals assayed in body fluid identifications and DNA typing for current and future analyses. Storage conditions for all types of evidence present must be considered so that the evidence is not compromised. In addition to the storage requirements detailed in the Quality Manual, the following procedures will be followed:

Sexual assault kits may be stored in a cooler, freezer, or dry area at room temperature once received in the laboratory. After analyses, sexual assault kits may be stored at room temperature. Sexual assault kits that contain blood or urine specimens are ideally stored in a refrigerator.

When applicable, wet evidence should be dried upon receipt. Refrigerate or freeze liquid whole blood specimens until a sample is dried on an appropriate substrate; be careful when frozen blood has thawed, as it may expand and cause vial breakage. Portions of blood standards submitted in liquid form should be dried on stain cards within 30 days of receipt by the assigned analyst. Once the sample is in dried form, the liquid blood tube will be placed back in its original packaging and returned to the property room.

Cases containing small, dry items may be stored at room temperature or frozen depending on available space. Larger items such as clothing, bedding, weapons, and other physical evidence containing bloodstains should be stored in a dry area at room temperature until examination.

Consumption of Evidence

The evidence's quality and quantity will be preserved as much as possible without sacrificing the quality of the analyses. Good judgment must be exercised to determine the smallest amount of sample that should be consumed for analysis that will still provide accurate results. Whenever possible, at least half of the evidence sample will be preserved for possible re-analysis (for both additional screening and additional DNA analysis). When this is not possible, the appropriate personnel (submitting officer, prosecuting attorney, and/or defense attorney) will be consulted and written permission will be obtained before the evidence is consumed. All evidentiary hairs are treated individually and cannot proceed to DNA analysis without consumption permission.

Chain of Custody

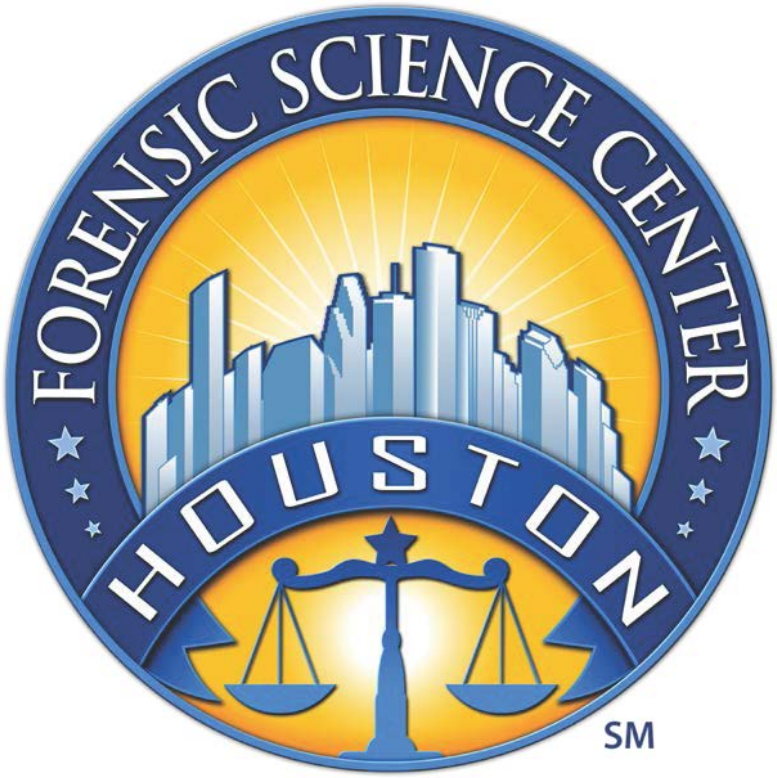
Refer to the Laboratory Quality Manual for chain of custody policies and procedures.

Reference Samples

Whenever possible, reference samples should be collected and retained from suspects, complainants, witnesses, consensual sex partners, and/or other individuals for the purposes of elimination. Oral swabs are the preferred reference sample for the Laboratory, but blood is certainly a viable source of reference material. Anecdotally, Sexual Assault Nurse Examiner (SANE) protocol dictates that evidentiary oral swabs are not only collected well before reference/known saliva swabs, but also after oral rinsing to help prevent possible foreign DNA from contaminating a reference/known saliva sample.

Potential for Contact DNA

Should contact DNA potentially be probative in a case, it is permissible for the examiner to bypass certain procedures that could possibly remove contact DNA that may be present. For example, an examiner may choose to skip the presumptive Acid Phosphatase press-out test and simply portion for the confirmatory microscopy examination in an effort to preserve potential contact DNA, if both penile and digital penetrations are alleged. Examiners may also choose to skip serological analysis entirely in an effort to maximize the available DNA. Case records must clearly document when and why a routine procedure such as the acid phosphatase or phenolphthalein press-out test is not performed.



Forensic Biology: Screening

Blood Detection

Forensic Analysis Division

6 Blood Detection

Please refer to SOP #4 (Reagent Quality Control) for quality control testing, storage, labeling, and expiration guidelines, of the Phenolphthalein Test Kit, Luminol and ABAcard® HemaTrace® Test Devices.

Many samples submitted to the Crime Laboratory are tested for the presence of blood. Generally, a sample will be subjected to a presumptive phenolphthalein test. If positive, the sample will be subjected to a confirmatory test for the presence of human blood. Testing is typically discontinued on phenolphthalein negative samples.

6.1 Phenolphthalein Test Kit

Scope

Catalytic tests for blood are based on the peroxidase-like activity exhibited by the heme group of hemoglobin. The test is exceedingly sensitive to minute traces of hemoglobin and its derivatives but will produce a false positive reaction in the presence of any of a number of oxidizing substances. Should a color reaction take place, the result only indicates the possible presence of blood; the test is therefore a presumptive test.

Safety

This reagent presents the following hazards:

Solution B: Flammable. Handle with care. Harmful or fatal if swallowed, inhaled, or absorbed through skin. Avoid contact with eyes, skin, and clothing. Light sensitive! Avoid light, heat, sparks, and flame. Keep container tightly closed.

Solution C: Handle with care. May be harmful if swallowed, inhaled, or absorbed through skin. Avoid contact with eyes, skin, and clothing. Avoid heat, sparks, and flame. Keep container tightly closed.

Wear gloves, lab coat, and mask during preparation and use. Broken skin should be covered.

Equipment, Materials, and Reagents

- Sterile diH_2O
- Sterile cotton swab(s) or filter paper
- Phenolphthalein Forensic Test reagents* - Solutions B and C

* Solution A is also included in the Phenolphthalein Forensic Test. However previous testing showed that using sterile diH_2O in place of Solution A did not affect the outcome of this test. Therefore, sterile diH_2O will be used in place of Solution A.

Procedure

1. Moisten a sterile cotton swab or piece of filter paper with sterile diH_2O**.**
2. Rub or press down on the suspected bloodstain with a sterile cotton swab or filter paper to allow for any possible blood to transfer.
3. Apply approximately 1 drop of Solution B to the swab or filter paper.

4. Observe briefly to identify color change. A green color is often observed when blood is present; any other color change should be documented.
5. Add approximately 1 drop of Solution C to the swab or filter paper.
6. Observe the swab or filter paper for an immediate pink color change indicating a positive reaction.
7. After interpretation, mark the items/areas tested with the results.
8. Discard the swab or filter paper by placing it in the biohazard trash.

Interpretation

Appearance of an immediate pink color change after the application of Solution C is a presumptive positive result for blood. No color change indicates a presumptive negative result for blood. A negative result indicates that blood is absent or below the detection threshold.

Substrate controls may be included in body fluid identification tests as appropriate.

6.2 Luminol

Scope

Catalytic tests for blood are based on the peroxidase-like activity exhibited by the heme group of hemoglobin. In the case of luminol, the catalytic oxidation of the substrate compound produces light. Because the reagents are applied as a spray over a large area, the test is primarily used in the visualization of bloodstain patterns. As with similar presumptive blood tests, a number of oxidizing substances will produce a false positive reaction. Should a positive reaction take place, the result only suggests the presence of blood; the test is therefore a presumptive test.

Safety

This reagent presents the following hazards: luminol is an irritant. Sodium perborate and sodium carbonate are toxic and irritating. Avoid breathing dust; do not get in eyes, on skin, or on clothing. Avoid breathing sprayed solution. Wash hands after handling. Gloves must be worn during testing. Broken skin should be covered.

Equipment and Supplies

- Luminol powder: 3-aminophthalhydrazide
- Sodium carbonate
- Sodium perborate
- Sterile diH_2O
- Two darkened or foil wrapped spray bottles
- Scale

Preparation (Reagent A)

1. Measure 250 mL of sterile diH_2O.
2. Add 2.5 g of sodium carbonate.
3. Mix until all of the powder is dissolved.

4. Add 0.5 g of luminol powder.
5. Mix until all of the powder is dissolved.

Preparation (Reagent B)

1. Measure 250 mL of sterile diH_2O.
2. Add 6.5 g of sodium perborate**.**
3. Mix until all of the powder is dissolved.

Procedure

1. Mix equal volumes of Reagents A and B to create a luminol mixture. The mixture is unstable and must be prepared fresh immediately before use; it is only stable for about one hour.
2. Darken the examination room as completely as possible.
3. Spray luminol mixture onto evidence and observe for luminescence (spraying will deposit a light white film on surfaces).
4. Document or photograph luminescent areas.
5. Re-spray as necessary. The ability to further test the stain decreases with increased or repeated spraying. Excessive spraying will cause stains to run, therefore, excessive spraying of non-porous surfaces is not suggested.
6. Dispose of excess solution in the regular sink, flushing with water.

Interpretation

Faint to strong luminescence shows oxidation of the luminol reagent and represents a positive presumptive result for blood. A negative result (a lack of luminescence) indicates that blood is absent or below the detection threshold.

Substrate controls may be included in body fluid identification tests as appropriate.

6.3 ABAcard® HemaTrace® Test Devices

Scope

The ABAcard® HemaTrace® test is a qualitative detection method specifically designed for forensic identification of human blood. Heme is a part of the hemoglobin molecule that is characteristic of red blood cells found in blood. The ABAcard® HemaTrace® has been shown to detect as little as 0.05 µg/ml of hemoglobin in 10 minutes.

The sample is added to a sample well where any detectable human hemoglobin (hHb) present in the sample will bind with mobile monoclonal antihuman Hb antibody which has an attached pink dye particle. The resultant mobile antigen-antibody complex migrates through an absorbent strip to an area where an immobile polyclonal antihuman Hb antibody is bound. The mobile antigen-antibody complex binds to the immobile antibody creating an antibody-antigen-antibody sandwich. Conjugated pink dye particles become visible in the area of immobilized antibody (in the test area: “T”) when the hHb concentration in the sample exceeds 0.05 µg/ml. The resultant

pink band indicates a positive result. As an internal positive control, hHb antibody-dye conjugates cannot bind to the antibody in the test area "T", but are captured by an immobilized anti immunoglobulin antibody present in the control area "C" forming a complex. The captured pink dye particles will thus form a band in the control area "C", indicating that the test has worked properly and that appropriate procedures have been followed.

Hemoglobin from the Family Mustelidae (ferrets) and higher primates also gives a positive result. The examiner must evaluate the likelihood of blood from either of these sources being present when this test is used as, forensically, the practical implications of this cross reactivity is minimal.

The ABAcard® HemaTrace® test may be skipped if, in the analyst's opinion, consumption for human origin testing will reduce the potential success of subsequent DNA analysis. Samples that are of limited size may be documented as Quantity Not Sufficient For Further Analysis ("QNS"). Only a presumptive screening result will be reported but DNA analysis may be performed.

Equipment, Materials, and Reagents

- ABAcard® HemaTrace® Test device – one per sample
- ABAcard® HemaTrace® extraction buffer
- Pipettes and pipette tips
- Timer

Procedure

1. Allow the sample(s) to warm to room temperature if they have been refrigerated.
2. Extract body fluid by placing suspected bloodstain sample into the extraction buffer provided with the kit and allow to sit for 5-30 minutes. Aged samples may be less soluble, and should therefore be soaked closer to 30 minutes. The extract(s) may be placed at room temperature in an ultrasonic cleaner for 5-15 minutes. The analyst must exercise good judgment regarding the amount of sample to be portioned for each ABAcard® HemaTrace® test device, providing sufficient sample for subsequent DNA analysis. Stain intensity and size are a few characteristics that should be considered. Generally, up to approximately ¼ of a swab or up to approximately $(1\ cm)^2$ of a stain may be consumed for this analysis.
3. For each sample, unwrap and label an ABAcard® HemaTrace® test device.
4. Add 150 µl of supernatant to the sample well "S" of the device.
5. Results may be read up to 10 minutes from application of the supernatant. Positive results can be seen as early as 2 minutes.
6. Record the ABAcard® HemaTrace® test results on the Serology Results worksheet.
7. Have a second qualified analyst examine the ABAcard® HemaTrace® test device and verify the documented test result(s) by initialing and dating the appropriate Serology Results worksheet.

Interpretation

There are two lines that appear on the test device:

1. **Control "C" line:** the presence of this line confirms the integrity of the test components and the test procedure. It is expected for all tests and must be present. Absence of the "C" line is an invalid result and the test should be repeated if adequate sample remains.
2. **Test "T" line:** A line indicates the presence of human hemoglobin and should be documented as a positive result. The absence of a line or a line that appears after 10 minutes is a negative result. A negative result indicates that blood is absent or below the detection threshold.

Substrate controls may be included in body fluid identification tests as appropriate.

Note: the presence of the "High Dose Hook Effect" may give false negative results due to the presence of a high concentration of human hemoglobin in the sample. When huge amounts of human Hb bind to the antibody to form an antigen-antibody complex but also free Hb migrates towards the test area "T", the antibody in the test area "T" is blocked by this free Hb. As a result, the mobile antigen-antibody complex with the pink color cannot bind to the antibody and the pink line cannot form in the test area "T", even though a lot of Hb is actually present. In such cases where human blood is strongly suspected, the extract may be retested using a 1:10 or 1:100 fold dilution.

6.4 References

1. Abacus Diagnostics, Inc. ABAcard® HemaTrace® For the Forensic Identification of Human Blood Technical Information Sheet.

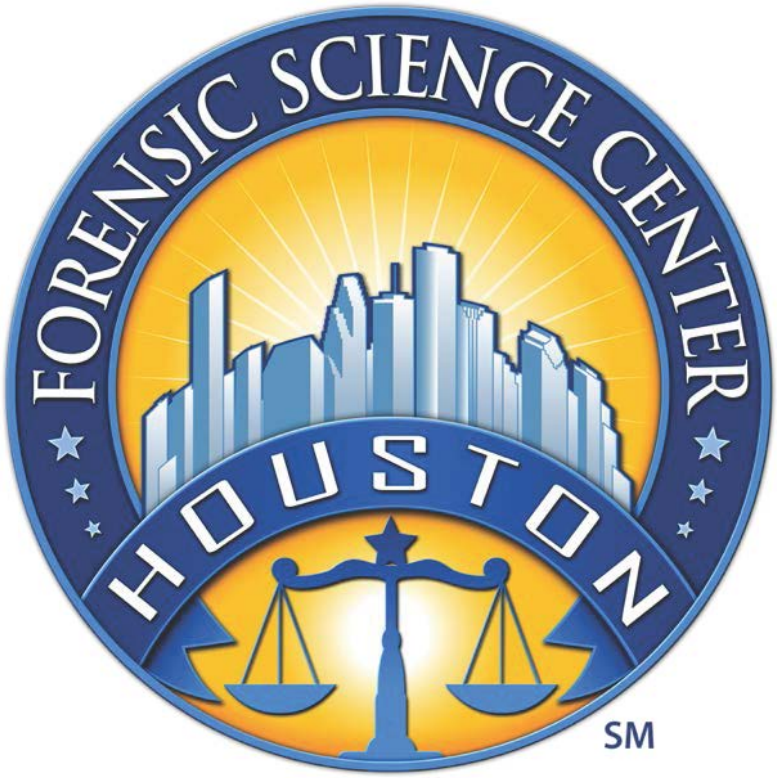
2. Gaensslen, Robert E. 1983. *Sourcebook in Forensic Serology, Immunology, and Biochemistry.* U.S. Department of Justice, National Institute of Justice. Sections 10.3.2 and 10.3.3.

3. Lee, Henry C. 1982. Identification and grouping of bloodstains. *In*: Forensic Science Handbook, Volume 1. Richard Saferstein, ed. Prentice-Hall, Inc., Englewood Cliffs, New Jersey. Chapter 7, p. 273.

4. Lytle, L. T. 1978. Chemiluminescence in the visualization of forensic bloodstains. Journal of Forensic Sciences 23(3): 550-562.

5. Saferstein, R. *Forensic Science Handbook: Identification and Grouping of Bloodstains*, (Prentice-Hall, Inc., 1982), p. 274.

6. Stoilovic, M. 1991. Detection of semen and blood stains using Polilight as a light source. *Forensic Science International* 51: 289-296.



Forensic Biology: Screening

Semen Detection

Forensic Analysis Division

7 Semen Detection

Please refer to SOP #4 (Reagent Quality Control) for quality control testing, storage, labeling, and expiration guidelines, of the Acid Phosphatase Spot Test, Alternate Light Source, SERI Christmas Tree Stain, Phosphate Buffered Saline (1X), and SERATEC® PSA Semiquant test devices.

Many samples submitted to the Crime Laboratory are tested for the presence of semen. Generally, a sample will be subjected to a presumptive acid phosphatase test, followed by a confirmatory microscopic examination for the presence of spermatozoa. If the sample is not microscopically positive, a third test, the test for the presence of prostate-specific antigen, may be warranted and executed.

7.1 Alternate Light Source

Scope

The Alternate Light Source (ALS) lamps within this lab will be used for detection of forensic semen stains. These lamps provide intense light of specific wavelengths through a hand-held wand.

Some dried semen stains on cloth are detectable visually because their color, off-white or yellow, is different from that of the material on which the semen has been deposited. However, on some substrates, semen stains are not readily visible. Under intense blue light (~450 nm), semen stains typically fluoresce. Blood absorbs light and will not fluoresce; fibers and other bodily fluids can typically fluoresce. Should fluorescence take place, the result only suggests the presence of semen; the test is therefore a presumptive test.

Equipment, Materials, and Reagents

- Alternate light source (ALS) set at 450-455 nm.
- Orange and/or yellow glasses

Procedure

1. Turn on power switch and ensure the fans are working.
2. Wear the proper glasses given and darken the examination room.
3. Turn on lamp switch.
4. Direct the ALS at the evidence samples.
5. Document areas of apparent fluorescence on the evidence.

When ready to shut down the machine, turn off lamp switch and wait for the unit to cool down (~5 minutes). Then turn off the power switch.

Interpretation

Should a stain fluoresce, the result only indicates the possible presence of semen.

Substrate controls may be included in body fluid identification tests as appropriate.

7.2 Acid Phosphatase Spot Test

Scope

Acid phosphatase (AP) is found in relatively large quantities in semen and its presence is indicative of the possible presence of semen in a stain. In the following procedure, acid phosphatase is detected by a color-change reaction. Acid phosphatase liberates the phosphate from α-naphthyl phosphate, and the released naphthol combines with tetrazotized o-dianisidine to form a purple azo dye.

The AP test is semi-quantitative. A stronger reaction is more likely to indicate semen. However, because acid phosphatase occurs in other body fluids, most notably vaginal secretions, this is only a presumptive test. The presence of semen in the sample can subsequently be confirmed by the presence of spermatozoa.

Equipment, Materials, and Reagents

- Sterile diH_2O
- Sterile cotton swab(s) or filter paper
- AP Spot Test reagent
- Timer
- Non-porous surface
- Clips (optional)

AP Procedure for Swabs

Note: Begin at step 1 when it is necessary to transfer the suspected semen sample from an item of evidence to a cotton swab or swabs for ease of testing. Otherwise, begin at step 3 following the procedure below:

1. Moisten cotton swab(s) with sterile diH_2O.
2. Swab the suspected semen stain from the evidence.
3. Moisten filter paper with sterile diH_2O.
4. Press the swabs firmly onto the filter paper.
5. Apply one or two drops of the AP Spot Test reagent to the filter paper.
6. Observe the treated filter paper for up to 60 seconds for a color change.
7. After interpretation, document the test results onto the serology worksheet(s).
8. Discard used filter paper by placing it in the biohazard trash.

AP Mapping Procedure

This procedure can be used when larger items of evidence are presented.

1. Spread the clothing item, bedding, towel, or other item flat onto a non-porous surface (use clips if necessary).
2. Moisten filter paper with sterile diH_2O.
3. Lay the moist filter paper over the item or area of the item to be tested. Press the paper firmly against the item for approximately 30 seconds to 5 minutes to allow for any possible acid phosphatase to transfer to the paper.
4. Mark the position of the paper on the item.
5. Remove the filter paper.
6. Spray or apply drop(s) of AP test reagent to the filter paper.
7. Observe the treated filter paper for up to 60 seconds for a color change.
8. After interpretation, mark the tested areas with results.
9. Discard used filter paper by placing it in the biohazard trash.

Interpretation

Should a color reaction take place within 60 seconds, the result only indicates the possible presence of semen; the test is therefore a presumptive test.

Grade the reaction according to the time it takes for the color to appear as follows:

- 0-15 seconds: 4+
- 16-30 seconds: 3+
- 31-45 seconds: 2+
- 46-60 seconds: 1+
- No change, or color change after more than 60 seconds: negative

A negative result indicates acid phosphatase is absent or below the detection threshold.

Substrate controls may be included in body fluid identification tests as appropriate.

7.3 Body Fluid Extraction

Scope

Possible body fluid stains are removed from the substrate and dissolved in PBS for testing. The extract is used to collect cellular debris from a stain for spermatozoa examination and can also be used for p30 testing.

Microscopic examination for spermatozoa or p30 testing may be skipped if, in the analyst's opinion, consumption for these tests will reduce the potential success of subsequent DNA analysis. Samples that are of limited size may be documented as Quantity Not Sufficient for Further Analysis ("QNS"). Only a presumptive screening result will be reported but DNA analysis may be performed.

Equipment, Materials, and Reagents

- Microcentrifuge tube(s)

- Forceps (optional)
- Scalpel and/or scissors
- Mini-centrifuge
- 1X PBS
- Pipette and pipette tips
- Ultrasonic cleaner

Procedure

1. Add sample to a microcentrifuge tube. The analyst must exercise good judgment regarding the amount of sample to be portioned for stain extraction, providing sufficient sample for DNA analysis. Stain intensity and size are a few characteristics that should be considered. Generally, up to approximately ¼ of a swab or up to approximately $(1\ cm)^2$ of a stain may be consumed for this procedure.
2. Add approximately 200 µl of 1X PBS to the sample. More PBS may be required to ensure the sample is fully submerged.
3. The extract(s) may be placed at room temperature in an ultrasonic cleaner for 5-15 minutes. Alternatively, the extracts may be soaked for 30 minutes - 24 hours in refrigerated storage.
4. Centrifuge the extract(s) briefly.
5. The extract, containing any cellular debris, will be used for microscopic spermatozoa examination and may also be used for p30 testing.
6. Store the extract refrigerated, when necessary, for up to 24 hours. Beyond 24 hours the extract should be stored frozen.

Substrate controls may be included in body fluid identification tests as appropriate.

7.4 Microscopic Spermatozoa Examination

Scope

Spermatozoa (sperm) detected on an evidence sample confirm the presence of semen; the test is therefore a confirmatory test. This procedure uses the SERI Christmas Tree Stain which is a Kernechtrot-Picroindigocarmine differential biological stain that assists in scanning slides for the presence of spermatozoa. The test consists of a two stain method which dyes the tip of sperm heads pink, the bottom of sperm heads red, the tails green or blue and any epithelium green with red nuclei.

Human sperm are flagellated with a total length of about 50 µm. The sperm cell head generally is oval, flattened at the anterior end, with dimensions about 4.6 µm x 2.6 µm x 1.5µm.

Identifying characteristics for spermatozoa:

1. Size (head is approximately 4.6 μm x 2.6 μm x 1.5μm)
2. Morphology (oval to round or teardrop-shaped structure)
3. Acrosomal cap at tip of sperm (unstained or lightly stained)
4. Staining (dark pink to red head, with unstained or lightly stained acrosomal cap; tails green or blue, if present)
5. Presence of a flagella or tail (may not be present)

EQUIPMENT, MATERIALS, AND REAGENTS

- Microscope slide
- Body fluid extract
- Pipette and pipette tips
- Heat block
- Christmas Tree Stain - Solution A and Solution B
- Timer
- Tap water
- 95% ethanol
- Compound microscope
- Immersion oil

Procedure

If slide has already been prepared, proceed to step #4.

1. Pipette 10 µl of the supernatant from the body fluid extract onto a glass slide. Fix smear by heating the slide on the heat block for approximately 5 minutes.
2. Stain the slide.
    a. Cover the sample area on the slide with Solution A for 10 minutes.
    b. Wash with tap water by gentle flooding.
    c. Cover the sample area with Solution B for 15 seconds.
    d. Wash with tap water by gentle flooding.

e. Flood the slide with 95% ethanol and allow the slide to dry for at least 5 minutes on the heat block.

3. Examine the slide for the presence of spermatozoa at 1000X magnification.
4. Document the approximate number of sperm seen on the slide. Once the count exceeds 25 spermatozoa observed, the documented count can be "25+", ">25", or "TNTC". The presence of only one spermatozoon must be verified by a second qualified analyst who must initial and date the examination documentation.
5. If too much cellular material and/or debris is potentially obscuring any spermatozoa on the slide for the analyst to make a determination about the presence or absence of sperm, the following procedure may be performed:
    a. Centrifuge the sample to pellet any cells. Remove and save supernatant for p30 analysis.
    b. Wash the cell pellet 1x with 500µl of sterile diH_2O or 1X PBS, pipetting the pellet up and down or vortexing.
    c. Centrifuge again to pellet any cells.
    d. Re-suspend in 50 to 300µl of sterile diH_2O or 1X PBS, again by pipetting up and down or vortexing.
        a. Use the new suspension to make another slide, repeating steps 2-5. Examination documentation must reflect the creation of multiple slides.

INTERPRETATION

Sperm heads stain red and the tails stain green by this procedure. A minimum of one sperm head should be identified to confirm the presence of semen. A search of the entire slide is required before negative results for spermatozoa can be reported. If the slide contains too much cellular material or debris for the analyst to determine the presence or absence of sperm, the analyst may then report the result as "inconclusive". Alternatively, if sperm-like objects are observed, but one or more of the identifying characteristics are lacking, the slide may be reported as "inconclusive".

It is recommended that "inconclusive" samples proceed to DNA analysis, along with positive samples.

7.5 SERATEC® PSA (p30) Semiquant Test

Scope

The cells that line the ducts of the prostate make a glycoprotein known as p30 or prostate-specific antigen (PSA). The protein is secreted into seminal fluid to a concentration of approximately 0.2 - 3.0 mg/ml, and its detection may indicate the presence of semen. PSA may be found at very low concentrations in vaginal fluid (0.4-1.25 ng/ml) and is therefore a presumptive test.

The SERATEC® PSA Semiquant test is a detection method specifically designed for forensic identification of p30. This test will be used qualitatively only in this laboratory. The test is capable of detecting p30 in a concentration range of at least 2ng/ml - 100μg/ml PSA.

SERATEC® PSA Semiquant test contains two monoclonal murine anti-PSA antibodies as active compounds. One of these antibodies is immobilized at the test region on the membrane. The upstream control region and the region of the internal standard (between control and test region) contain immobilized polyclonal goat anti-mouse antibodies. The amount of antibody at the internal standard is adjusted to a color intensity of the line, which is equal to the color intensity of the test line at a PSA concentration of 4 ng/ml. A glass fiber pad downstream of the membrane is used for sample loading and transmission to a second fiber pad with the dried and gold-labeled second monoclonal murine anti-PSA antibody. PSA at the sample will bind to the remobilized gold-labeled antibody and form a PSA-gold-labeled-anti-PSA-antibody complex.

Through the capillary effect of the membrane, the reaction mixture including the complex mixture is carried upwards with the fluid. In any case, the colored gold-labeled anti-PSA-antibody will bind to the anti-mouse-antibody at the control region and the region of the internal standard, thus developing two red lines (one at the control region and one at the region of the internal standard). These two lines are independent of the existence of PSA in the sample and indicate only the correct execution of the test.

If the sample contains PSA, the PSA-gold-labeled- anti-PSA-antibody complex will bind to the immobilized monoclonal antibody of the test result region that recognizes another epitope on the PSA molecule (sandwich complex). The binding is indicated by the formation of an additional line. Thus a PSA positive sample will show three colored lines in the result window.

Equipment, Materials, and Reagents

1. SERATEC® PSA Semiquant test device – one per sample
2. Body fluid extract
3. Pipettes and pipette tips
4. Timer

Procedure

1. Allow previously extracted sample(s) to warm to room temperature if they have been refrigerated.
2. For each sample, unwrap and label a SERATEC® PSA Semiquant test device.
3. Add 120 μl of the supernatant to the sample well of the device.
4. Results may be read up to 10 minutes from application of the supernatant.
5. Record the SERATEC® PSA Semiquant test results on the Serology Results worksheet.
6. Have a second qualified analyst examine the SERATEC® PSA Semiquant test device and verify the documented test result(s) by initialing and dating the appropriate Serology Results worksheet.

Interpretation

There are three lines that appear in the test device “result window”:

1. **Control "C" line:** the presence of this line confirms the integrity of the test components and the test procedure. It is expected for all tests and must be present. Absence of the "C" line is an invalid result and the test should be repeated if adequate sample remains.
2. **Internal Standard line:** The intensity of this line correlates with a p30 concentration of approximately 4 ng/ml. It is expected for all tests and must be present. The absence of the internal standard line is also an invalid result and the test should be repeated if adequate sample remains.
3. **Test "T" line:** A line indicates the presence of semen and should be documented as a positive result. The absence of a line or a line that appears after 10 minutes is a negative result or p30 concentration is below the detection limit.

Samples that produce a negative result but show strong positive acid phosphatase activity must be diluted to an appropriate dilution and be re-tested to ensure the High Dose Hook Effect is not occurring (see SOP #6.3 ABAcard® HemaTrace® Test Devices).

7.6 Guidelines for Sexual Assault Cases When Contact or Digital Penetration is Alleged

It is not possible to anticipate every situation that may arise or to prescribe a specific course of action for every case; therefore, the examiner must exercise good judgment based on experience and common sense. The following are intended only as guidelines for cases in which digital penetration or other contact may have occurred. Specific case information may suggest a course of action other than what is prescribed below.

Competent (ex: can clearly recall incident):

External swabs with clear notes on why it was collected (ex: "he kissed my breast"):

- Bathed: NTC, add bathed statement in report
- Not bathed: retain

External swabs without clear notes on why it was collected (outer envelope only states "Breast swabs"):

- Press-out; do not retain negatives

Intimate swabs:

Penile and other penetration:

- Within 24 hours, snippet test; retain regardless of semen results
- Beyond 24 hours, press-out; do not retain negatives

Non-penile penetration:

- Within 24 hours, retain
- Beyond 24 hours, NTC

Incompetent (ex: mentally challenged/infants/children):

- Snippet test, retain regardless of semen results

Unconscious (ex: doesn't know what happened, drunk/drugged):

- External swabs:
  - Bathed: NTC, add bathed statement in report
  - Not bathed: retain
- Intimate swabs:
  - Press-out; do not retain negatives

- Unless specifically states a digital/contact situation:
  - Within 24 hours, snippet test; retain regardless of semen results
  - Beyond 24 hours, press-out; do not retain negatives

Any information indicating that semen may be present should always be considered and samples processed accordingly, regardless of above guidelines (ex: “he ejaculated on my neck”, shorts, etc.).

7.7 References

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2. http://www.historyforkids.org/scienceforkids/biology/cells/transportation2.htm. Accessed July 21, 2012.

3. Edwin L. Jones, Jr. (2005). The Identification of Semen and Other Body Fluids. In R. Saferstein (Ed.), *Forensic Science Handbook, Volume II*. (pp. 329-399). Mt. Laurel, New Jersey: Pearson/Prentice Hall.

4. Baechtel, S. F. 1988. The identification and individualization of semen stains. *In*: Forensic Science Handbook, Volume 2. Richard Saferstein, ed. Prentice-hall, Inc., Englewood Cliffs, New Jersey. Chapter 7, p. 349.

5. Gaensslen, Robert E. 1983. *Sourcebook in Forensic Serology, Immunology, and Biochemistry.* U.S. Department of Justice, National Institute of Justice. Sections 10.3.2 and 10.3.3

6. Kind, Stuart S. 1957. The use of acid phosphatase in searching for seminal stains. Journal of Criminal Law, Criminology, and Police Science. 47(5):597-600

7. Omniprint™ 1000A-110 Operating Instructions

8. SERATEC® PSA Semiquant Test. *In-vitro diagnostic test for professional forensic use for the detection of seminal fluid by the semi-quantitative determination of PSA (Prostate-specific antigen).* Product insert, revised June, 2011.

9. Smith, Charles. 1979. An acid phosphatase reagent for use in searching large areas for seminal stains. Unpublished

10. Stoilovic, M. 1991. Detection of semen and blood stains using Polilight as a light source. *Forensic Science International* 51: 289-296.

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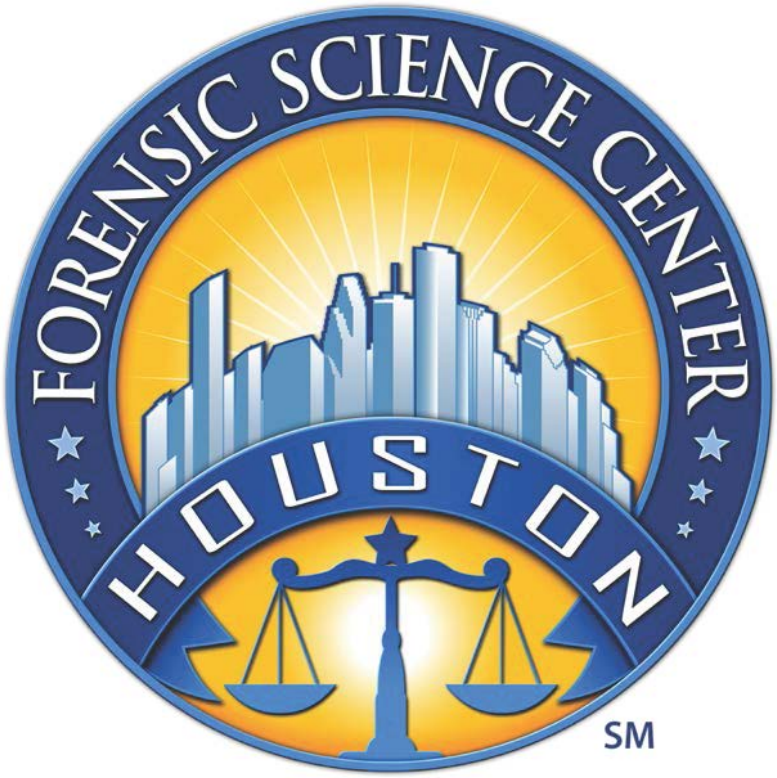


Forensic Biology: Screening
Trace Observation/Collection

Forensic Analysis Division

8 Trace Observation/Collection

8.1 Scope

This procedure may be used when necessary for the preservation of trace evidence prior to forensic biology analysis. Examples of typical trace evidence in criminal cases include fingerprints, glove prints, hairs, cosmetics, plant fibers, mineral fibers, synthetic fibers, glass, paint chips, soils, botanical materials, gunshot residue, explosives residue, and volatile hydrocarbons (arson evidence). However, the reporting of trace may be limited to apparent hairs and fibers.

The Locard Exchange Principle presumes that when a person comes into contact with an object or person, a cross-transfer of material, sometimes trace evidence, occurs. While this laboratory does not currently examine trace evidence, it does make every effort to collect and preserve it for potential future analysis. To do so, trace evidence is generally collected prior to the commencement of biological analysis.

Dedicated trace collection room doors should remain closed at all times. The rooms will be vacuumed and the floors damp mopped at least once per week when in use. Lab coats will be provided in the trace rooms for analysis. Lab coats used in other areas of the laboratory will not be taken into the trace rooms due to possible contamination. For any method used, tables and tools will be cleaned with bleach solution (or other lab-approved cleaning solution) before each use to remove any extraneous debris. At a minimum, tools will be cleaned and new bench paper will be used for each item when multiple items are received packaged together.

Generally, for any method used, a clean sheet of butcher paper will be used on the table for each evidence package opened.

Evidence from victim(s) and suspect(s) will be examined in separate rooms to prevent cross contamination. The examiner will change lab coats between the examination of items from victim(s) and suspect(s). If separate rooms are not available, items from victim(s) and suspect(s) can be processed in the same room after a period of 7 days.

8.2 Procedure

Equipment, Materials, and Reagents

- Butcher paper
- Tweezers
- Plastic zipper bags
- Clear packing tape or trace evidence lifters

There are several options available for ensuring that trace evidence is properly collected/stored/preserved. These are as follows:

1. Visual examination: locate and remove trace evidence from the evidence item with tweezers. Recovered trace will be placed in a suitable labeled container (zipper bags or on tape) to prevent loss or contamination of the sample.

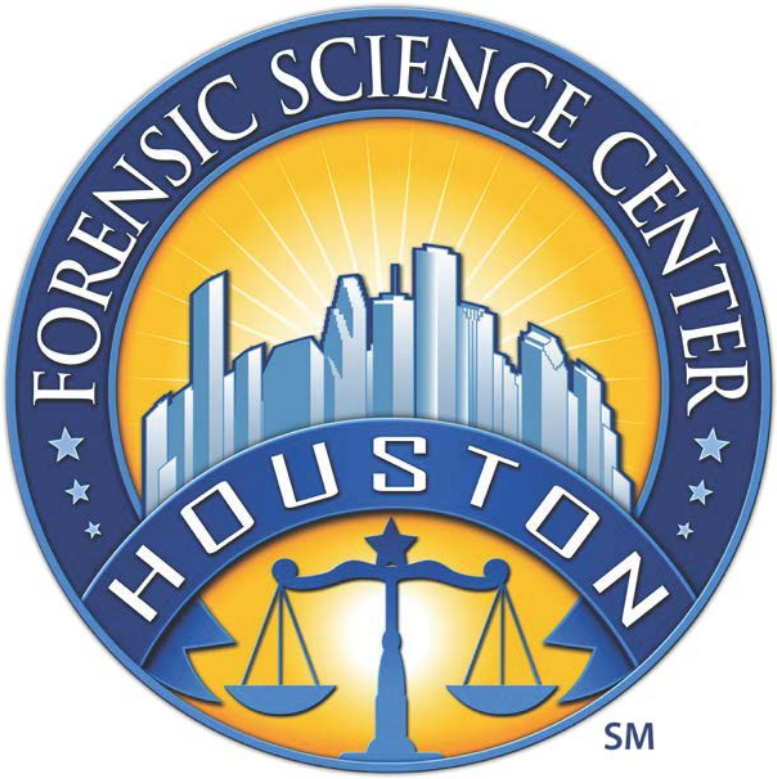
2. Tape Lifting: clear tape is pressed over the area potentially bearing trace evidence. This application is repeated until all areas of interest are covered. New tape should be used once the tape in current use loses its stickiness. Tape(s) will be placed on the inside surface of a clean cut open zipper bag or a sheet protector. The bag/protector will be labeled as to its contents.
3. Retaining in butcher paper: Trace evidence may also be preserved by retaining the evidence item in butcher paper. In some instances, however, it may be most appropriate to repackage the item using the original packaging. For example, a swab with an embedded hair or fiber may be repackaged in the original swab carton. The method of packaging selected should prevent damage, deterioration, and/or loss of the trace evidence. The evidence item may only be re-opened in a clean trace environment to prevent contamination and/or loss.

Place all collected trace evidence in the original evidence container with the item from which it was collected.

The observation or collection of trace evidence must be documented on the appropriate examination documentation. Examiners should not make any attempt to identify on their worksheets what was collected (i.e. hair, fiber, etc) unless they also possess current training in the disciplines of Trace Evidence. However, it is permissible for sample descriptions to include adjectives such as “apparent” or “-like” when describing trace evidence (e.g., hair-like or apparent fibers).

8.3 Reference

1. Saferstein, R. (2007). *Criminalistics: An Introduction to Forensic Science, Ninth Edition.* Upper Saddle River, New Jersey: Pearson/Prentice Hall.



Forensic Biology: Screening
Contact DNA Collection

Forensic Analysis Division

9 Contact DNA Collection

9.1 Scope

This procedure may be used, when necessary, for the collection of contact DNA evidence prior to forensic biology analysis. This laboratory does not currently "screen" for the presence of contact DNA through a method such as microscopic examination for the presence of epithelial cells. Contact DNA is generally very limited in nature and consumption during screening may further reduce the potential success during DNA analysis. For this reason, when an item is suspected of containing contact DNA, efforts to collect possible contact DNA will be made. These samples may then be submitted for DNA analysis.

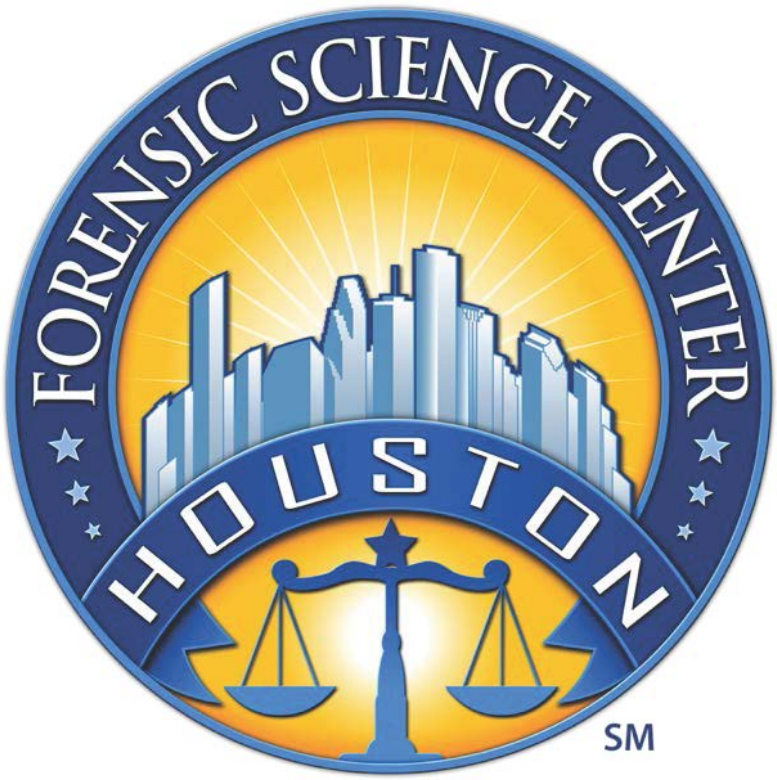
9.2 Equipment, Materials, and Reagents

- Sterile cotton swabs
- Sterile reagent grade water
- Swab drying box (optional)

9.3 Procedure

1. Moisten swab(s) with sterile diH_2O.
2. Swab the suspected area(s) on the evidence with the tip(s) of the cotton swab(s). Efforts to concentrate the possible contact DNA on the tip of the swab should be made. This will hopefully serve to increase chances of DNA success not only by concentrating the possible DNA but also in the absence of visible staining, later portioning will more likely capture DNA if the correct area of the swab is selected for portioning. As the area to be swabbed increases in size, so too may the number of swabs needed to adequately collect the evidence.
3. The wet swab may be followed by a dry swab on non-absorbent surfaces.
4. Avoid any suspected semen or blood stains. These sources of biological material generally contain more DNA and will potentially "mask" any lower level contact DNA. These stains may be processed separately.
5. Allow swab(s) to completely dry in a swab drying box for 30 minutes. Alternatively, the swab(s) may be air dried overnight in a secure and clean environment.

Sometimes, such as in the case of fingernail scrapings and clippings, suspected bloodstains cannot always be avoided. In such instances, the analyst will look for any visible red/brown staining and document accordingly prior to swabbing the item. It may be appropriate to forgo blood or semen detection in order to maximize potential contact DNA for DNA anlaysis.



Forensic Biology: Screening Portioning for DNA Analysis

Forensic Analysis Division

10 Portioning for DNA Analysis

10.1 Scope

After serological analysis, any evidence that will be retained in the laboratory can be portioned for potential DNA analysis. Please refer to SOP #5 (Evidence Evaluation and Handling) for guidelines on general evidence handling, and the consumption of evidence in particular. These should be treated as guidelines only; good judgment and case specifics may warrant a deviation from what is prescribed below.

10.2 Equipment, Materials, and Reagents

- Scalpel or scissors
- Microcentrifuge tube(s)
- Weighing paper or butcher paper

10.3 Procedure

NOTE: Make every effort to preserve at least half of the sample for re-analysis. If this is unavoidable, written permission must be obtained.

1. Set the evidence onto paper.
2. Portion the evidence according to the chart below:

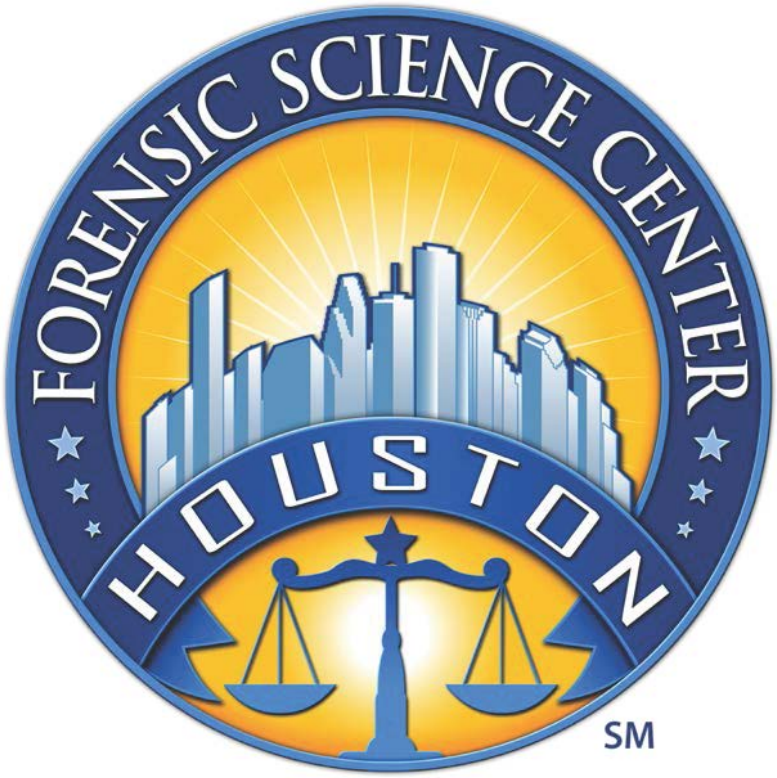
Swabs

Results	**Portions**
P30 (+) only, no spermatozoa	~ ½ of total swab(s)
<10 spermatozoa	~ ½ of total swab(s)
≥ 10 spermatozoa	Remainder of swab cut for p30 or micro
HemaTrace (+)	~ ½ remainder of swab cut for HemaTrace
QNS	~ ½ of total swab(s)
Contact	~ ½ of total swab(s)
Bloodstain card	~ 0.5 cm^2 from center of stain
Known saliva swabs	~ ½ of 1 swab

Clothing

Results	**Portions**
<10 spermatozoa or p30 (+) only	~ 1 cm^2 cutting
≥ 10 spermatozoa	~ 0.5 cm^2 cutting
HemaTrace (+)	~ 0.5 – 1 cm^2 cutting
QNS	~ ½ of the stain

3. Place portion into a microcentrifuge tube.
4. Label microcentrifuge tube with case number, item number, and initials.



Forensic Biology: Screening

Case Records

Forensic Analysis Division

11 Case Records

11.1 Examination Documentation

The notes and other documentation must support the conclusions of the examiner. The laboratory report must communicate both the analytical results and the conclusions of the examiner, conveying the essence of what he or she would say if asked for an expert opinion in court. Decisions may be made by police officers, attorneys and the courts based on the report alone without examiner clarification, so the report should be able to stand alone. Some case record requirements can be found in the Quality Manual, any additional case record requirements are included here.

Refer to the quality manual for chain-of-custody policies and procedures, and documentation of chain-of-custody, as well as documentation required in all Laboratory case records.

Documentation must be in such a form that another qualified examiner or supervisor, in the absence of the primary examiner, would be able to evaluate what was done and interpret the data. The reviewer of the case should be able to determine from the notes that sufficient testing, relevant testing, and correct methods of testing were used. To this end, all documentation of procedures, standards and controls used, observations made, results of tests performed, charts, graphs, photographs, sketches, etc. that are used to support the examiner's conclusions must be preserved as a record. Observations, data, and calculations shall be recorded at the time they are made; the date of an in-house photograph shall be included on the photograph or associated examination documentation. Examination records shall be of a permanent nature. If a written examination record is created (or if original observations are made) on non-traditional media (for example: sticky notes, paper towels, gloves, etc.), then either the original media will be retained or an electronic scan/picture of the original will be retained in the case record. Once an electronic scan/picture is created, the original hardcopy may be destroyed. Examination documentation should reflect the name and/or initials of the individual who performed the work.

Appropriately completed SOP worksheets should be used during the analyses. Examination documents should have notes that help in the identification of the item of evidence. A written description may suffice for some items, whereas others may need a drawing, sketch, or photograph.

If an item is submitted for immediate analysis, such as a mobile phone that will be swabbed for possible contact DNA and immediately returned to the submitting party, it is not necessary for the evidence to be sealed and it may not be possible to establish a proper seal and/or to be marked with a unique identifier. However, case record documentation must include identifying information that will permit a later identification of the item handled. For example, in the absence of analyst markings on the actual item of evidence, a photograph of the mobile phone's serial number, along with a photo of the mobile phone, will enable later identification of the actual item handled.

Items collected at autopsy do not always include the name of the complainant, as it may be unknown at the time of the autopsy. If morgue evidence is received without the name of the complainant, the evidence should be described using the Medico-Legal number (ML#) and/or "unknown". Alternatively, if written notice is provided by the investigator or other case agent, the complainant name provided may be used in the evidence descriptions, in conjunction with the

ML#. This written notice must be maintained in the case file and may be referred to in the report.

Each examination worksheet(s) should include the following: initials, case number, controls, dates of analyses, item names/numbers/quantity, results, and disposition. Items that are not tested or opened should be described to the extent to which they can be. A written description may suffice for some items, whereas others may need a drawing or photograph. Descriptions of evidence should be clear and sufficient to permit the later identification of an item. They may include any of the following: tears, cuts, missing buttons, possible bullet holes, location, and length of possible stab holes, soiling condition, degree of degradation, type of material, significant foreign material, etc. Significant stains must be drawn or documented in a manner which clearly demonstrates the location, relationship to other stains, reactions to screening tests, etc. Abbreviations may be used; see SOP #12 for a listing of approved abbreviations.

If needed, the location of the substrate blank control and its relationship to the body fluid stain in question must be clearly documented. The type of material or fabric the stain is on may be significant.

11.2 Reports

Report the results of any tests performed. If presumptive tests are positive but confirmatory tests are not performed, this fact should be reported. For some items, it may be useful to report what area of the item was tested. Also include trace examination, whether collected or observed. Instructions should be included in the report as to requesting trace analysis if needed. Note if any items listed were not analyzed.

It may be appropriate to include in the report a request for the submission of known samples from any of the following: suspects, complainants, witnesses, consensual sex partners, and/or other individuals for the purposes of elimination.

Although interpretation of individual tests are discussed in the appropriate protocols, analysis results must be considered together to ensure that conclusions take into account all reasonable possibilities. During analysis, the examiner must continuously monitor results for problems and inconsistencies that may ultimately affect final conclusions. Therefore, careful review of notes and results by the examiner should be undertaken before conclusions are drawn. A supervisor or qualified examiner may assist in the developing of the appropriate conclusions. Slightly different situations may lead to different conclusions. When in doubt, consult with other qualified examiners and/or the supervisor.

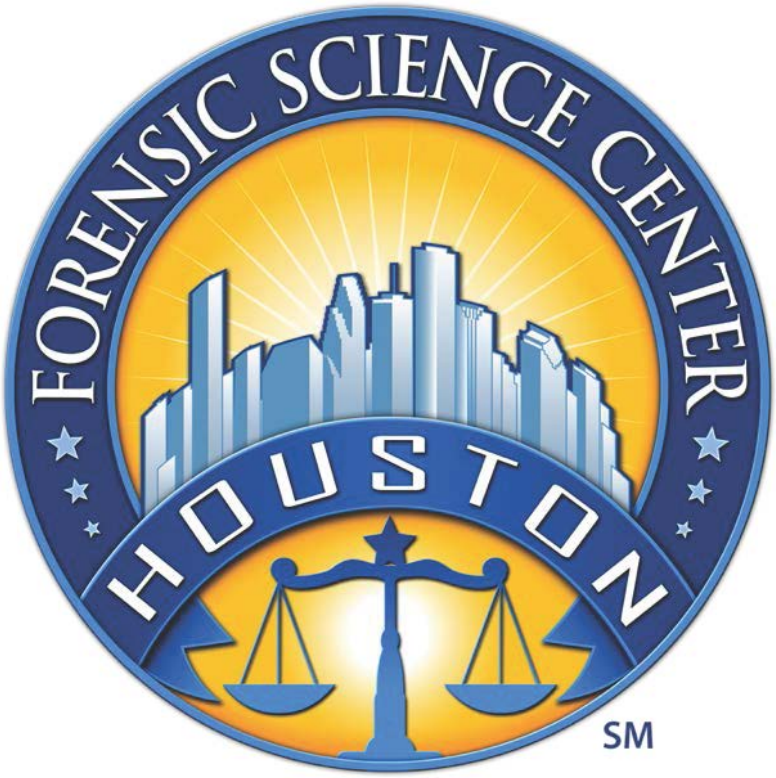
Reports will include the disposition of all items of evidence, whether they are retained within the laboratory or returned to the submitting agency.

11.3 Examination Counts

Information related to the number of examinations performed during each analysis can be documented on the “Serology Examination Count Sheet” in LIMS. This information can be used by laboratory management to accurately capture metrics such as laboratory capacity, volume of specific requests, and sectional productivity.

The following is a summary of how totals for this form should be calculated:

Category	**How to Calculate**
Number of Items	Number of items listed on the report
ALS Exams	Number of items viewed with ALS (excluding controls)
AP	Number of swabs/stains tested (excluding controls)
Sperm searches	Number of slides viewed
p30	Number of cards tested
PHT	Number of swabs/stains tested (excluding controls)
HemaTrace	Number of cards tested
Trace collection/observation	Number of items with trace collected or trace observed
Bloodstain cards	Number of bloodstain cards made
Swabbings/cuttings	Number of cuttings or swabs collected
Portions for DNA	Number of portions created
Total examinations	Total of examinations – LIMS will calculate



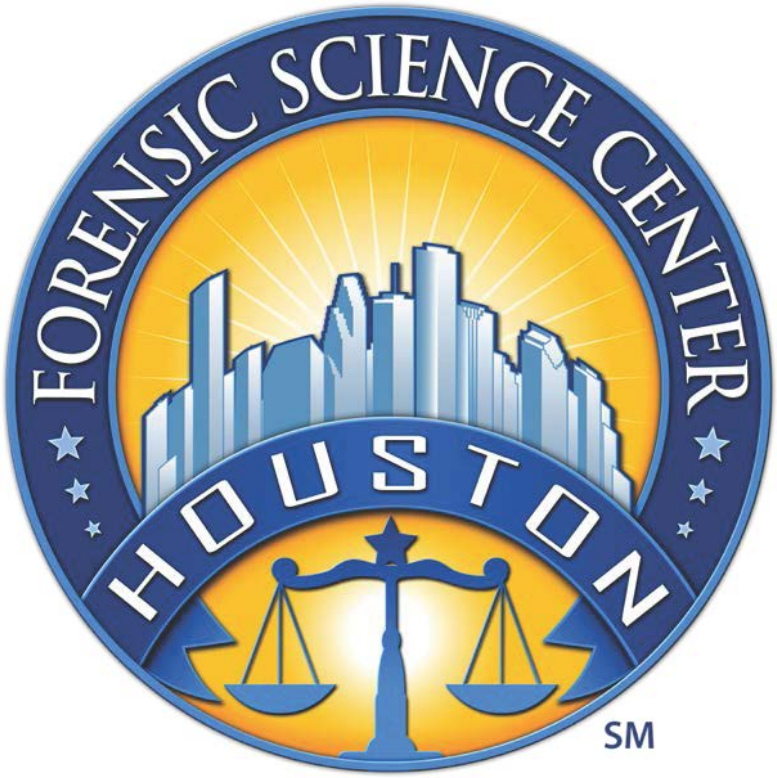
Forensic Biology: Screening Abbreviations

Forensic Analysis Division

12 Abbreviations

In addition to standard scientific abbreviations, as well as those found in the dictionary, the following abbreviations are defined here and acceptable for use on case work documentation. Non-standard scientific abbreviations, those not found in the dictionary, and abbreviations not contained on this list must be defined on each examination document on which it is used.

Ø	none	FNSC	fingernail scrapings/clippings	PR	HPD Property Room
~	approximately	FTC	found to contain	PR#	payroll number
ALS	alternate light source	HPS	hospital patient sticker	PREP	preparation
AP	acid phosphatase	HRS	hours	PT	patient
APP	apparent	HT	HemaTrace®	Q	questioned sample
AR	administrative review	INC	inconclusive	QNS	quantity not sufficient (for further analysis)
BC	barcode	INC #	Incident Number	R/B	reddish/brown
BSC	bloodstain card	IPC	Internal positive or PCR control	REP	representative
BUC	buccal	IT	item	REC'D	received
c̄	containing	K	known standard sample	RMS	Records Management System
CAPT	captain	KBS	known buccal swabs	RPR	return to property room
COMM	communication	KSS	Known saliva swabs	S/SEC	second
COMPL	complainant	LG	large	SA	sexual assault
CONT'D	continued	LIMS	Laboratory Information Management System	S-CELLS	sperm cells
CS	Controlled Substances	LT	lieutenant	SCRN	Screening
CSP	Consensual sex partner	MED	medium	SF	sperm fraction
CTRL	control	MICRO	sperm search by microscopy	SGT	sergeant
D	depleted	MFG	manufacturer	SM	small
DOB	date of birth	N/A	not applicable	ST	stain
diH_2O	deionized water	NEG	negative	STC	said to contain
DISP	disposition	NSO	no stains observed	SUPPL	supplement
ECD	excessive cellular debris	NTC	no testing conducted	SUSP	suspect
E-CELLS	epithelial cells	NTO	no trace observed	SWIFS	Southwestern Institute of Forensic Sciences
EF	epithelial fraction	NSTO	no significant trace observed	TEMP	temperature
EMS	Evidence Management System	NVB	no visible bloodstain	TNTC	too numerous to count
ENV	envelope	OFTC	opened, found to contain	TOX	Toxicology
EtOH	ethanol	OLO	online offense system	TR	technical review
EVID	evidence	PBS	phosphate buffered saline	UNK	unknown
EXP	expiration	PG/PP	page/pages	VAG	vaginal
F	frozen	PHT	phenolphthalein	W/	with
FA	Firearms	PKGD/PKG'D	packaged	WIF	walk-in freezer
FCN	Forensic case number	POS/+	positive	YOA	years of age
FNS	fingernail scrapings	POSS	possible		



Forensic Biology: Screening
Chelex Extraction of DNA

Forensic Analysis Division

13 Chelex Extraction of DNA

This procedure will allow for the extraction of DNA from samples containing semen. The Chelex extraction is an efficient and quick method to obtain the DNA from the samples. The addition of DTT will allow for the digestion of samples that contain semen. Samples may or may not contain semen.

Safety

Body fluids, tissues, and extracts may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Gloves must be worn during testing. Clothing may protect unbroken skin; broken skin should be covered.

Standards, Control, and Calibration

One reagent blank must be processed for each extraction batch as a negative control.

Please refer to SOP #5 (Evidence Evaluation and Handling) for guidelines on general evidence handling, and the consumption of evidence in particular.

Equipment, Materials, and Reagents

- scissors, scalpel, tweezers
- 5% Chelex
- Proteinase K (10 mg/ml)
- ~1 M DTT
- tubes – 1.5 ml, microcentrifuge
- microcentrifuge with rotor for 2 ml tubes at room temperature
- water bath or dry bath ~56.0 °C
- water bath or dry bath ~100.0 °C
- vortex
- stir plate

Procedure

Portioning

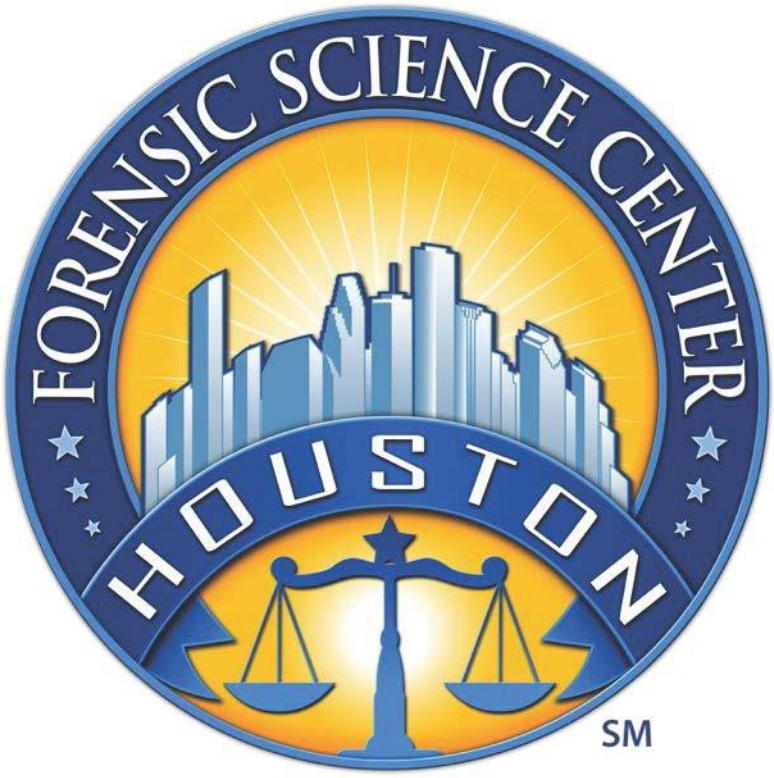
A portion of all swabs provided will be taken for Chelex extraction.

1. Set the evidence onto paper.
2. Portion ~1/8 of all swabs for a particular piece of evidence.
3. Place all portions into a microcentrifuge tube.
4. Label microcentrifuge tube with case number, item number, and initials.

Chelex Extraction

1. Make sure Chelex beads are evenly distributed in solution.
    a. If necessary, re-cap and mix stock by inversion periodically during processing.
    b. Alternatively, chelex solution may be made in a sterile bottle and a reagent stir bar used to keep the beads distributed.
2. Add approximately 200 uL of 5% Chelex solution.

   a. Ensure the substrate is completely saturated. Additional Chelex solution may be needed to completely submerge the swab.
   b. If additional Chelex is needed, the same volume must be added to reagent blank
3. Add 10 uL of Proteinase K and 15.6 uL of ~1M DTT
4. Vortex sample for ~10 seconds.
5. Incubate at ~56.0 °C for 30-45 minute.
6. Vortex
7. Incubate at ~100 °C for 8 minutes. Take care to ensure that microcentrifuge tube lids remain closed during the ~100 °C incubation.
8. Briefly vortex, then centrifuge the tube to pull beads to the bottom of the tube.
   a. Chelex beads and substrates should be pulled to the bottom of the tube. If a supernatant layer is not visible then centrifuge again. A higher centrifugation setting may be necessary. A pipette tip may be used to force the substrate down prior to centrifugation.
   b. Alternatively, if substrate will not stay in the bottom of the tube, use a large bore pipette to remove at least 75 ul of supernatant to a new tube. Centrifuge the new tube to pellet any beads. Continue with step 9.
9. Carefully transfer ~50 uL of supernatant into a new 1.5ml microcentrifuge tube. Be sure to not transfer any Chelex beads.
   a. If any of the beads are transferred, pipette the supernatant back into the Chelex solution. Repeat steps 7 – 9. Use a new final tube if supernatant is added back to the Chelex solution.
10. Store samples refrigerated (up to 2 weeks) or frozen (>2 weeks) until ready to perform Plexor HY analysis.



Forensic Biology: Screening
Plexor HY Quantification

Forensic Analysis Division

14 Plexor HY Quantification

The Promega Plexor HY kit contains all necessary reagents for the simultaneous real-time PCR amplification, detection, and quantification of human autosomal and human male DNA in a sample. The results can be used to determine the presence of male DNA in samples with overwhelming female DNA.

The DNA quantification assay combines three separate assays:

- A multicopy human autosomal DNA assay
- A multicopy human male DNA assay
- An internal PCR control assay

The kit works with the Applied Biosystems 7500 Real-Time PCR System and SDS Software v1.2.3 for data collection. Data analysis is accomplished by exporting the data to the Plexor Analysis Software v1.5.6.7.

Safety
Body fluids, tissues, and extracts may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Gloves should be worn during testing. Clothing may protect unbroken skin; broken skin should be covered.

Kit Contents and Storage
The Plexor HY kit comes in two sizes, 200 and 800 reactions with a 20 µL reaction volume. Store the entire kit at -15°C to -25°C upon receipt. Store the male genomic DNA standard at 2°C to 8°C after first thaw, the remaining kit should continue to be stored at -15°C to -25°C between uses.

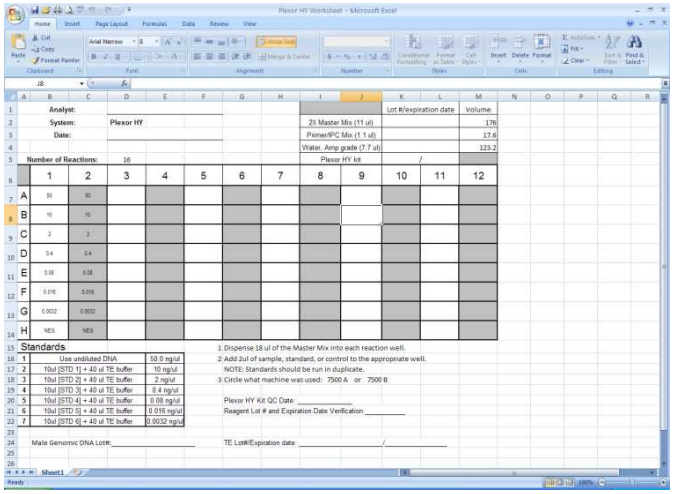
Plexor® HY System 200 reactions
Product Size Cat. # DC1001
Includes:
• 2 × 1ml Plexor® HY 2X Master Mix
• 200µl Plexor® HY 20X Primer/IPC Mix
• 150µl Plexor® HY Male Genomic DNA Standard, 50ng/µl
• 2 × 1.25ml Water, Amplification Grade

Plexor® HY System 800 reactions
Product Size Cat. # DC1000
Includes:
• 8 × 1ml Plexor® HY 2X Master Mix
• 4× 200µl Plexor® HY 20X Primer/IPC Mix
• 3 × 150µl Plexor® HY Male Genomic DNA Standard, 50ng/µl
• 5 × 1.25ml Water, Amplification Grade

NOTE: Keep Primer/IPC Mix and Master Mix protected from direct exposure to light. Excessive exposure to light may affect the fluorescent dyes.

Creating a Plexor HY worksheet

1. Open the "Plexor HY Worksheet" template (an Excel spreadsheet).
2. Type sample names into the sample sheet. Ensure each standard is present and in duplicate.
3. Print the file for documentation in the case file(s).


Plexor HY Worksheet - Microsoft Excel
Analyst:
System:
Plexor HY
Date:
Number of Reactions:
16
2X Master Mix (11 ul)
Primer/IPC Mix (1.1 ul)
Water, Amp grade (7.7 ul)
Plexor HY kit
Lot #/expiration date
Volume
176
17.6
123.2
Standards
1. Dispense 18 ul of the Master Mix into each reaction well.
2. Add 2ul of sample, standard, or control to the appropriate well.
NOTE: Standards should be run in duplicate.
3. Circle what machine was used: 7500 A or 7500 B
Plexor HY Kit QC Date
Reagent Lot # and Expiration Date Verification
Male Genomic DNA Lot#:
TE Lot#/Expiration date:

4. Save the excel file with initials and date of run (i.e. AB092012)
5. Close excel.

Preparing the DNA Quantification Standards

Make a standard curve dilution series consisting of:

Standard	ng/µL
Male Genomic DNA Standard	50
B	10
C	2
D	0.4
E	0.08
F	0.016
G	0.0032

To create this series of standards, perform the following tasks:

1. Label 6 microcentrifuge tubes B, C, D, E, F, and G.
2. Dispense 40 µL of TE into the six microcentrifuge tubes.
3. Vortex the Male Genomic DNA Standard for 3 to 5 seconds
    a. Allow the Male standard to warm to room temperature.
    b. If this is the first use of the standard, thaw the DNA.
4. Prepare Standards B through G:
    a. Pipette 10 µL of the Male Genomic DNA Standard to the tube for Standard B.
    b. Mix the dilution thoroughly
    c. Using a new pipette tip, add 10 µL of the prepared standard to the tube for the next standard (e.g. 10 µL of Standard B into the tube for Standard C; the resulting mixture will contain 10 µL of the previous standard and 40 µL of TE)
    d. Mix the dilution thoroughly
    e. Repeat steps 4c and 4d until the dilution series is complete

NOTE: Any unused standard can be labeled with the analyst's initials and date of creation (e.g. ABC010101) and stored at 2°C to 8°C for up to two weeks.

Reaction and Plate Setup Procedure

1. Allow the Plexor HY kit components to thaw to room temperature. Mix the 2X Master Mix and 20X Primer/IPC Mix by brief vortexing.
2. Make a set of positive standards as described in the Previous section (entitled "Preparing the DNA Quantification Standards").
    a. If standards exist and are less than two weeks old, you may use them instead of making new standards.
3. Prepare a Master Mix consisting of the following reagents and volumes:
    a. The “Plexor HY Worksheet” will include the necessary volumes for the master mix
    b. The worksheet includes a correction factor for pipetting loss

Component	Volume Per Reaction (µL)
Plexor HY 2X Master Mix	11
Plexor HY 20X Primer/IPC Mix	1.1
Water, Amplification Grade	7.7

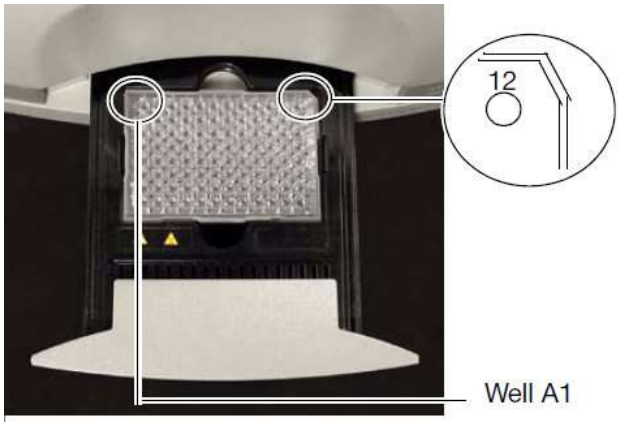
4. Vortex the Master Mix for 3 to 5 seconds, then briefly centrifuge the tube.
5. Dispense 18 µL of the Master Mix into each reaction well.
    a. Make sure the plate is correctly oriented, A1 will be to the top left

6. Add 2 µL of each sample, standard, or control (TE for negative control) into the applicable wells.
7. Seal the reaction plate with the adhesive cover.
8. Centrifuge the plate at ~3000 rpm for about 20 seconds in a plate centrifuge with plate holders to remove any bubbles.
9. Load the plate into the plate holder of the 7500. Ensure that the plate is correctly aligned in the holder.

Push the tray door to open it.

Insert the plate into the plate holder.

Load standard 96-well plates with the notched A12 position at the top-right of the tray.



Close the tray door.

Starting the 7500 Real-Time PCR System
Overview
Starting the 7500 Real-Time PCR System involves:

1. Starting the Computer, if not already on
2. Powering on the Instrument
3. Starting the 7500 SDS Software

Starting the Computer

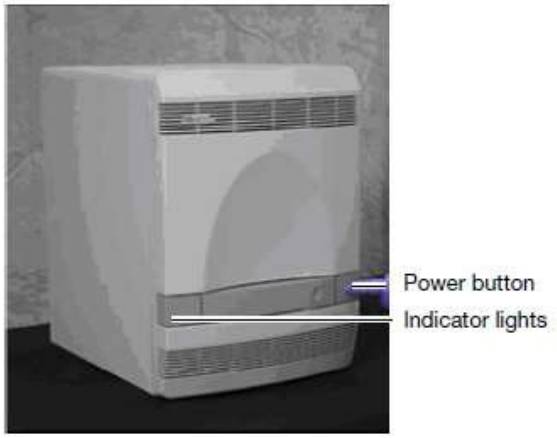
1. Open the laptop computer by pushing in the front center button, holding it, and lifting the lid.
2. Press the power button on the computer.
3. Enter the username associated with the computer, if applicable.
4. If required, type the corresponding password in the password field.

NOTE: Wait for the computer to finish starting up before powering on the 7500 instrument.

Powering on the Instrument

1. Press the power button on the lower right front of the 7500 instrument.
2. The indicator lights on the lower left of the front panel cycle through a power on sequence.
3. When the green power indicator is lit (not flashing), communication is established between the computer and the instrument.

NOTE: If the green power-on indicator is flashing or the red error indicator is lit, see the Applied Biosystems *7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide* (PN 4347828)



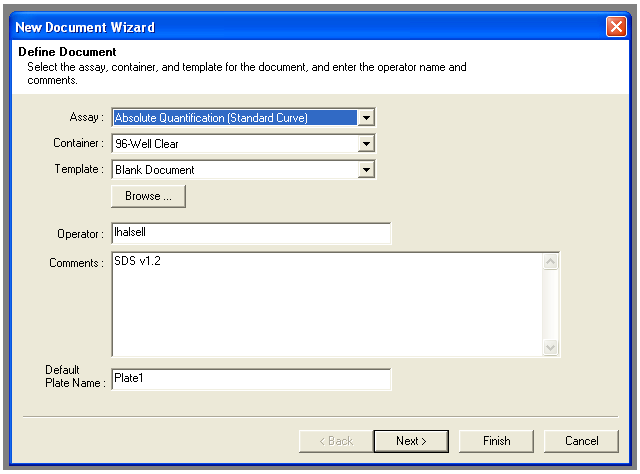
Starting the 7500 SDS Software

1. Start the **7500 System Software**. Double click on the software icon.

2. The software starts and displays the word "Disconnected" in the status bar on the bottom-right corner. The status changes to "Connected" only after the New Document Wizard is completed, the software is initialized, and the software is connected to the 7500 instrument. If the connection is successful, the software displays “Connected” in the status bar on the bottom-right corner.

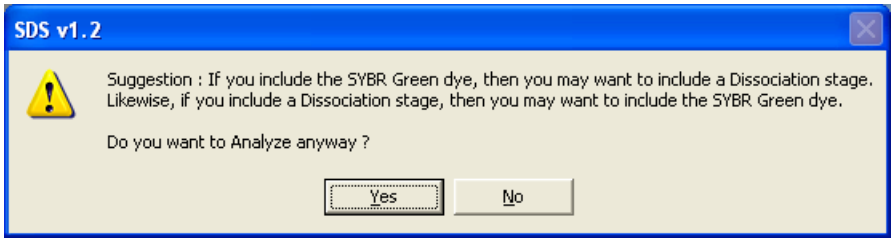
Creating a Plate Document and Starting the Run

1. Click **File > New**
2. Click **Browse** below the Template box.

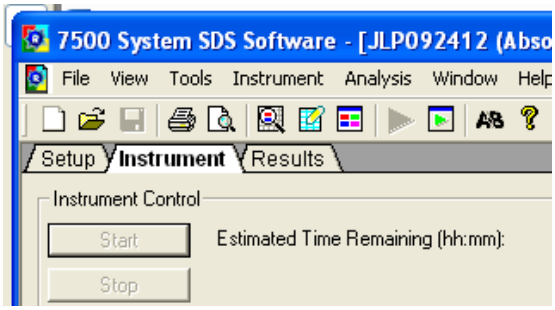


3. Navigate to the Desktop and select the “Plexor HY” template
4. Click **Open** (or double-click the template file)

NOTE: The following box will appear any time you open, close, save, or analyze the file. Always click yes to navigate pass the box.


SDS v1.2
Suggestion : If you include the SYBR Green dye, then you may want to include a Dissociation stage.
Likewise, if you include a Dissociation stage, then you may want to include the SYBR Green dye.
Do you want to Analyze anyway ?
Yes
No

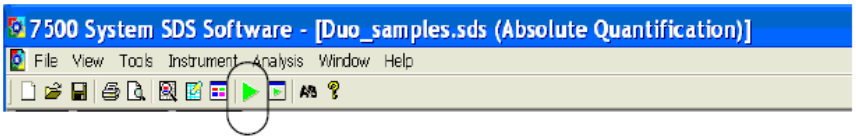
5. Click **File > Save As**
6. Name the run with initials and date (i.e. AB092012)
7. Save the file to the Desktop
8. Click on the Instruments tab and click **Start** to start the run.



9. Ensure that the **Estimated Time Remaining** shows a time before leaving the room.

Analyzing run and Exporting files with 7500 SDS Software

1. When the run is complete the instrument will display a successful run completion message.
2. Click okay
3. Omitting unused wells
    a. Select the unused wells according to your worksheet on the Results tab
    b. With the well selected, hit Ctrl + M
    c. The omitted wells will now show an "X" through them
4. On the menu bar, select **Analysis > Analyze**.
    a. Alternatively the green arrow in the menu can be clicked



5. Exporting Files
    a. Select **File > Export > Delta Rn**
    b. Save the file with the run name and the suffix "-delta" (i.e. AB092012-delta)
        i. Save the file to the Desktop with the run file
    c. Select **File > Export > Dissociation > Raw & Derivative data**
    d. Save the file with the run name and the suffix "-diss" (i.e. AB092012-diss)
        i. Save the file to the Desktop with the run file
    e. Do **NOT** open the export files. This will corrupt them for later use.
6. Close the 7500 run file and 7500 SDS software
    a. Click Yes to Save if prompted to do so.
7. Move the three saved files to the appropriate month and year folder of \\travfs01\CrimeLab\SHARED\BiologyInstruments\7500PCR\Plexor HY
8. Remove and discard the plate from the 7500
9. Turn off the 7500

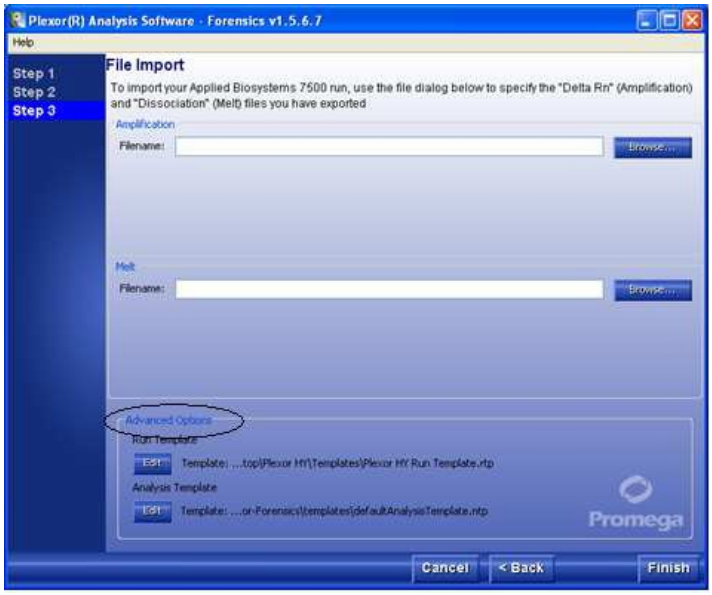
Plexor HY Analysis software

Note: The following instructions are suitable on software that has been appropriately initialized

Software set up and file import

1. Start the Plexor Analysis Software
2. Select **File > Import New Run**
3. Fill in the Assay Name with the same name as the 7500 run file (i.e. AB092012)
4. Make sure that the instrument is set to **Applied Biosystems 7500 SDS v1.4 & prior**
5. Click **Next**
6. Fill in the Operator Name, initials are acceptable
    a. The remaining fields can be left blank

7. Click **Next**
8. Click the upper **Browse** button to add the Amplification file
9. Navigate to the previously saved “Delta Rn” file (i.e. AB092012-delta)
10. Select file and click open
11. Click the lower **Browse** button to add the Melt file
12. Navigate to the previously saved “Raw & Derivative data” file (i.e. AB092012-diss)
13. Select file and click open
14. Under the **Advanced Options**
    a. Click **Edit** under **Analysis Template**
    b. Select **Defaults > Reset to Default**
    c. Click **OK**



15. Click Finish
16. A window will appear with the run data

Data Analysis and Report Generation

1. Make sure the **FL-Autosomal** Tab is selected
2. With all samples selected, go to **Edit > Add Standard Curve**
    a. Alternatively you can hit the “d” key. Caps lock cannot be on.
3. Select the **CO560-Y** Tab
4. With all samples selected, go to **Edit > Add Standard Curve**
    a. Alternatively you can hit the “d” key. Caps lock cannot be on.
5. Select the **Sample Names** Tab
    a. Open the Excel Plexor HY worksheet saved previously
    b. Select and Copy the wells that were used for the run
    c. Return to the Plexor Analysis software
6. Select **Edit > Paste Sample Names from Template**
    a. Alternatively you can hit the “Ctrl + t” key
7. Click **Accept Changes** in the bottom right of the window

8. Click on the **Standard Curves** Tab
    a. Evaluate the standard curves as follows
        i. Click on the “FL-Autosomal” tab
            1. The Autosomal curve, the slope must be between -3.1 to -3.7 and R2 ≥ 0.990
        ii. Click on the “CO560-Y” tab
            1. The Y curve, the slope must be between -3.0 to -3.6 and R2 ≥ 0.990

    NOTE: A limited number of outlier points may be omitted from the curve to bring these values within acceptable range. If these values are not within range, the procedure must be re-run. Removal of more than 3 data points must be approved by the Technical Leader. Points can be removed by changing the sample type under the “PCR Curves” tab. Refer to Plexor HY manual for more detail.
9. Select **Forensics > Set Normalization and IPC parameters**
    a. Do not change any settings
10. Click **Ok** on the window that pops up
    a. Give the software a minute and the window will change to the Forensic report tab under the Reports tab
11. Check that the first location is A1.
    a. Click on **Location** so the samples will be sorted by location
12. Select **File > Save Analysis File**
13. Title the file with initials and run date (i.e. AB092412)
14. Save the file to the appropriate month and year folder of \\travfs01\CrimeLab\SHARED\BiologyInstruments\7500PCR\Plexor HY

Data interpretation

1. Check that the **IPC Status** column is “OK” for all samples.

    NOTE: A “Check IPC” value indicates the possible presence of inhibitors. For any samples that say “Check IPC”, select that sample and examine the PCR curves under the PCR Curves tab. The absence of an IPC value under the CR610-IPC tab and a quantification value for a sample indicates either complete inhibition or a failure of the amplification reaction. The sample may be rerun. Alternatively, a dilution may be made and run again with Plexor HY. While the dilution would contain fewer inhibitors, it would also contain less DNA and may not be a true negative.

2. Check that the **Curve Status** column is “OK” for all samples.
    a. Depending on sample type (standard, non-template control, or sample) three other options may be displayed depending on sample type
        i. Check STD – displayed for samples designated as standard controls
        ii. Check NTC – displayed for samples designated as non-template controls

iii. Check Melts – displayed for sample designated as unknowns

NOTE: "Check" messages will only be displayed on samples that have concentration values. The message indicates that the melt curve needs to be assessed for that sample.

b. Examine the melt curves for samples that are **NOT** "OK".
   i. Melt curves need to be assessed for both Autosomal and Y results.
c. Melt curves can be examined under the "PCR Curves" tab. The melt curve is the bottom graph

NOTE: Melt curves are a measure of the PCR products produced during amplification. If a sample has a low level DNA concentration, but no melt curve, then the detected fluorescence is most likely from non-specific fluorescence and not from amplifiable DNA.

d. When examining the melt curves in the "PCR Curves" tab three different calls are available in the "Tm?" column on the right side
   i. Yes – the melt curve is within the designated range
   ii. No – the melt curve is not within the designated range
   iii. No Call – the melt curve is within the designated range, but there is insufficient amplification product to cross the threshold
e. Documentation of why melt curves were not "OK" will need to be made on the report print out.

3. Review raw data for all samples and controls. Examine amplification curves for unusual morphology. Samples with unusual curves may be re-run or reported as inconclusive.
   NOTE: It is recommended that the nearest standards above and below the unknown sample be used for comparison of curve morphology.
4. Return to the "Forensic" tab under the "Reports" tab
5. Check that all negative controls (including reagent blanks) have less than 5×10^{-3} (0.005) ng/µl of detectable DNA present. If negative control values exceed 0.005 ng/ul, then consult with the Technical Leader on available options.
6. Close the Plexor run file by **File > Close Current Analysis**.
   a. Alternatively you can click the red X in the upper right run file window.
   This is just above the printer icon.
7. Save results if asked to do so.
8. Reopen the file by **File > Open Analysis File**

a. Alternatively you can click the file icon in the menu bar
9. Navigate to the saved file and select it. Click **Open**
10. Return to the "Forensic" tab under the "Reports" tab if not already there
11. Make sure all samples are selected. All samples will be highlighted blue.
    a. All samples can be selected by clicking one sample and hitting Ctrl + A
12. Select the print icon on the right
13. Under Page Setup, change the orientation to Portrait
14. Click Print
15. Close the Plexor run file and software.
    a. Save any changes if prompted to do so
16. Add any comments to the report that are necessary. Examples include:
    a. If any points were dropped from the standard curve.
    b. Results of melt curve investigations
17. Place a copy of the report containing the results in the case file.

Reporting and Sample Retention

1. Samples that have less than 5×10^{-3} (0.005) ng/µl of detectable male DNA present shall be reported as inconclusive.
2. Inconclusive samples may proceed to DNA analysis depending on the availability of additional male DNA positive samples within a particular case.